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Abstract: Terrestrial plants have two to four times more ATP-binding cassette (ABC) transporter genes than other organisms, including their ancestral microalgae. Recent studies found that plants harboring mutations in these transporters exhibit dramatic phenotypes, many of which are related to developmental processes and functions necessary for life on dry land. These results suggest that ABC transporters multiplied during evolution and assumed novel functions that allowed plants to adapt to terrestrial environmental conditions. Examining the literature on plant ABC transporters from this viewpoint led us to propose that diverse ABC transporters enabled many unique and essential aspects of a terrestrial plant's lifestyle, by transporting various compounds across specific membranes of the plant.

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Plant ABC Transporters Enable Many Unique Aspects of a Terrestrial Plant's Lifestyle

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ABSTRACT

Terrestrial plants have two to four times more ATP-binding cassette (ABC) transporter genes than other organisms, including their ancestral microalgae. Recent studies found that plants harboring mutations in these transporters exhibit dramatic phenotypes, many of which are related to developmental processes and functions necessary for life on dry land. These results suggest that ABC transporters multiplied during evolution and assumed novel functions that allowed plants to adapt to terrestrial environmental conditions. Examining the literature on plant ABC transporters from this viewpoint led us to propose that diverse ABC transporters enabled many unique and essential aspects of a terrestrial plant's lifestyle, by transporting various compounds across specific membranes of the plant.

Key words: abscisic acid transporter, adaptation to dry land, ATP-binding cassette transporters, evolution, lifestyle of plants

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INTRODUCTION

ATP-Binding Cassette Transporters

ATP-binding cassette (ABC) proteins are powerful transporters that drive the exchange of compounds across many different biological membranes, in most cases against existing electrochemical gradients, using energy released from ATP hydrolysis (Wilkens, 2015). They are ancient and present in all organisms (Figure 1). Recent papers reported that ABC proteins transport coating materials, supportive materials, secondary metabolites, and plant hormones that regulate the overall development of plants. Interestingly, terrestrial plants have many more ABC transporter genes than higher animals (Figure 1 and Table 1). Based on a phylogenetic analysis of ABC proteins that have homology to a stress hormone abscisic acid (ABA) transporter, we propose that the ABC transporter genes might have undergone multiplication and diversification events during evolution that allowed plants to adapt to the land environment. We then describe the physiological roles of many individual transporters and discuss how their roles might have contributed to the establishment of traits necessary for survival on land. The traits we focus on here include secretion of surface coating materials, transportation of defense molecules, biochemical intermediate transport between organelles, hormonal transport, sequestration of xenobiotics to the vacuole, and seed germination control. Due to space constraints, this is not an exhaustive review; however, we hope that this work will provide an overview of the field of ABC transporters and inspire the reader to consider the relevance of ABC transporters to their own special interests.

TERRESTRIAL PLANT'S UNIQUE LIFESTYLE

Plants are highly specialized organisms that have many unique features that set them apart from other organisms, including their

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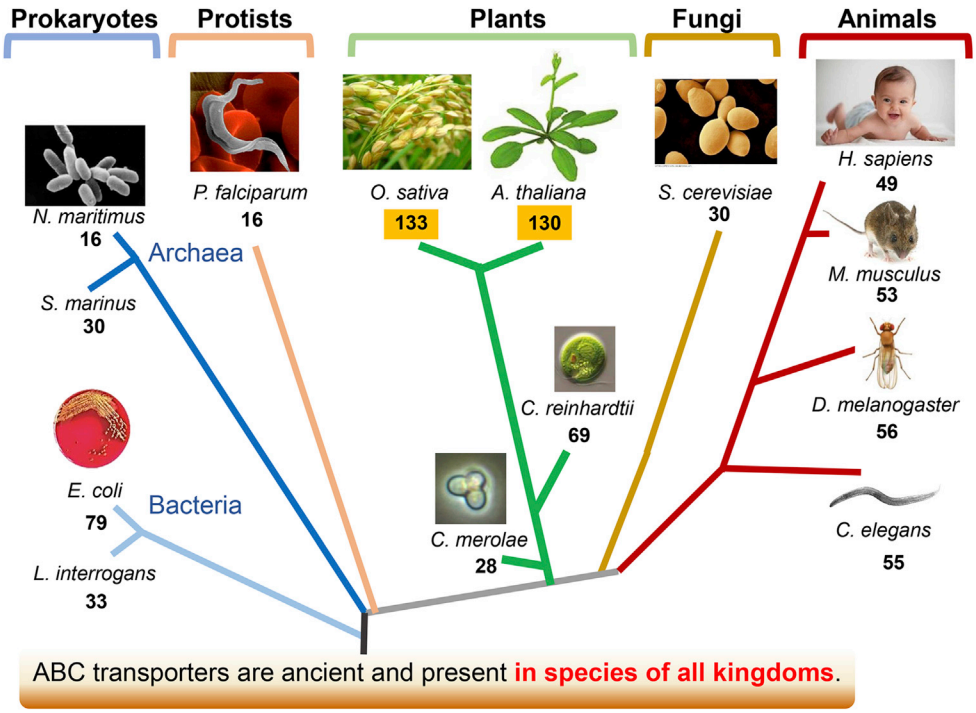

Figure 1. ABC Transporters Are Present in All Organisms and Are Especially Enriched in Plants.
Numbers below the species name indicate the numbers of ABC transporters in the organism. For example, rice has 133 different ABC transporters, while humans have 49. The number of ABC transporters in higher plant species is highlighted in yellow.

evolutionary ancestors, unicellular and multicellular microalgae. Plants developed wide surface areas for harvesting the light energy needed for photosynthesis, but wide surfaces increase the risk of desiccation and damage in dry and ultraviolet (UV) light-rich environments. Plants overcame this problem by coating their surfaces with cutin and wax layers and developing pigments that act as sunscreen. The wide surface area also restricted rapid movement, and might have been a factor in the adoption of a sessile lifestyle. The sessile lifestyle in turn might have shaped the unique defense systems of plants, which rely heavily on chemicals called secondary metabolites. Also, the body structure of plants is unique and effectively supports their body weight in the absence of the buoyancy of the aquatic environment. As an autotroph, plants synthesize many chemicals de novo, which necessitates the transport of intermediates from one organelle to another. Terrestrial plants developed organs that carry out different functions, which necessitated the transport of signaling molecules between the organs to coordinate physiological and developmental processes at the whole-plant level. Finally, many plants make seeds that can survive for years, until conditions are favorable for growth. Thus, the unique lifestyle of plants was most likely molded by the challenges they faced when changing their habitat from water to dry land.

It is tempting to speculate that during evolution of green plants from microalgae, ABC transporter genes underwent multiplication and functional diversification, and thereby assumed the ability to transport compounds critical for successful adaptation to dry land. These critical compounds include plant hormones (for coordinated growth and development), lignin precursors (for structural support), secondary metabolites (for defense), hydrophobic compounds (for surface coating), and diverse phenolics (for protection against UV light). As highlighted below, ABC proteins transport all of these compounds.

PLANTS HAVE A LARGE NUMBER OF ACTIVE TRANSPORTERS WHILE ANIMALS HAVE MORE ION CHANNELS

In general, organisms with more complex functions require a greater number of transporters with diverse functions to exchange metabolites and information between cells and organs. However, organisms living in different niches and with different response patterns might need different sets of transporters. We found that plants are enriched in ABC and secondary active transporters, whereas animals have more ion channels (Table 1). In other words, during the evolution of higher animals and plants, transporter genes were not simply multiplied in proportion to the increase in genome size, but distinct groups of transporter genes were expanded. It makes sense that plants have many secondary active transporters, as they are sessile organisms and most processes are rather slow. It also makes sense that animal cells developed many ion channels, since ion channels play important roles in nervous systems, which require many transporters with short response times and high capacities. Then, why do plants have many more ABC transporters than other organisms and, furthermore, why have plants developed specific sets of ABC transporters, as can be speculated by the proliferation of ABCB and ABCG transporters in terrestrial plants (Table 2)? To answer these questions, we

	Budding yeast	*Chlamydomonas*	Moss	Rice	*Arabidopsis*	*C. elegance*	Fruit fly	Mouse	Human
ABC transporters	**30**	**69**	**125**	**133**	**130**	**55**	**56**	**53**	**49**
P-type ATPases	16	26	43	43	46	22	19	44	45
Other ATP-dependent	30	29	80	63	78	3	20	70	41
Secondary transporters	228	288	565	843	769	348	351	484	447
Ion channels	20	66	93	131	149	234	157	394	391
Unclassified	1	4	47	4	53	0	6	34	40
Sum	325	482	953	1217	1225	662	609	1078	1013
Proteome size	5796	17 741	32 275	24 799	25 498	19 735	13 733	25 059	19 000

Table 1. Number of Transporters in Various Organisms.

Plants are enriched in ABC transporters (bold font) and secondary active transporters, while animals are enriched in ion channels. Transporter numbers are based on data from TransportDB (http://www.membranetransport.org/index.html, Ren et al., 2007) and information in the following review papers: yeast (Van Belle and Andre, 2001; De Hertogh et al., 2002; Brohee et al., 2010), *Chlamydomonas* (Merchant et al., 2007), moss (Lang et al., 2008; De Michele et al., 2012), rice (Baxter et al., 2003), and human (Almen et al., 2009; Ye et al., 2014). The proteome size is the estimated number of protein-coding genes reported in the following publications: budding yeast (Christie et al., 2009), *Chlamydomonas* (Blaby et al., 2014), *P. patens* (Zimmer et al., 2013), rice (Ouyang et al., 2007), *Arabidopsis* (Lamesch et al., 2012), *C. elegans* (Hillier et al., 2005), fruit fly (Clark et al., 2007), mouse (Bult et al., 2016), and human (Ezkurdia et al., 2014).

reviewed the functions of many plant ABC transporters and attempted to decipher their evolutionary paths, as explained below. The results indicate that, compared with animals, plant genomes are enriched in genes encoding ABC transporters, because these transporters are particularly suited to helping plants survive and be competitive under dry land conditions.

As a first step toward investigating the evolutionary history of higher plant ABCG genes, we constructed a phylogenetic tree based on the amino acid sequences of *Arabidopsis* ABCG40 (AtABCG40) and its homologs (Figure 2). We conducted a BLAST search to identify genes that encode amino acid sequences with close similarity to AtABCG40 in the genome sequences of two green algae, a moss, and a land plant. As sister taxa, two red algal species were used as outgroup. AtABCG40 is a specific transporter of ABA, a hormone important for tolerance to many different kinds of abiotic and biotic pathogen stresses that are commonly found in terrestrial environments. Although taxon sampling was limited, the tree (Figure 2) shows that moss (*Physcomitrella patens*) and flowering plants (*Arabidopsis thaliana*) share a common ancestor with marine (*Osteococcus taurus*) and freshwater (*Chlamydomonas reinhardtii*) green algae. Furthermore, the tree shows that land plants (moss and flowering plants) underwent explosive radiation of the ABCG40-like genes. Thus, it is highly likely that the number of ABCG40 homologs increased in a short period of time, allowing plants to adapt to the new land environment. Two other essential components of the ABA signaling machinery of land plants, i.e., orthologs of the PYR ABA receptor and ABA insensitive 5-binding proteins (AFPs), were identified only in land plants and not in green or red algae (Wang et al., 2015), consistent with this interpretation. Further analyses of the origin and evolution of ABC proteins of photosynthetic organisms, with expanded taxon sampling, might yield important insights into the evolution of land plants.

PLANT ABC TRANSPORTERS TRANSPORT CHEMICALS CRITICAL FOR ADAPTATION TO DRY LAND

Transport of Materials for Surface Coating

During evolution, terrestrial plants needed to adapt their architecture to limit desiccation in a dry environment. All aerial organs of land plants are covered with a hydrophobic cuticle layer that protects against biotic and abiotic stresses, including non-stomatal transpiration, fungal and bacterial pathogen attack, and UV exposure (Barthlott and Neinhuis, 1997; Krauss et al., 1997; Burghardt and Riederer, 2008; Müller, 2008). The cuticle is composed of highly lipophilic cutin and waxes. Cutin is the insoluble polymer matrix that overlays cell walls and serves as the main structural component of the cuticle. Cutin consists mainly of ω- and mid-chain hydroxy and epoxy C16 and C18 fatty acids esterified to each other and to glycerol (Nawrath, 2002; Heredia, 2003; Bonaventure et al., 2004). Cuticular waxes are mixtures of very-long-chain fatty acids and their derivatives, including secondary metabolites such as triterpenoids, phenylpropanoids, and flavonoids (Kunst and Samuels, 2003; Jetter et al., 2008). Because biosynthesis and modification of cuticular monomers occur mostly in the endoplasmic reticulum (ER) of the epidermis, the monomers must be transported from the ER to the plasma membrane and then across the plasma membrane. Many ABC transporters have been shown to transport hydrophobic coating materials, and all of these ABC transporters identified to date localize to the plasma membrane. Perhaps no ABC transporters that transport coating materials have been found at organellar membranes because multiple transporters and/or pathways that transport such materials from the ER to the plasma membrane exist.

CER5 (ABCG12) was the first ABC transporter identified as being involved in moving cuticular lipids (Pighin et al., 2004). The *abcg12/cer5/wbc12* stem epidermis has a significantly

Common name	Budding yeast	*Chlamydomonas*	Moss	Spikemoss	Rice	Thale cress	Roundworm	Fruit fly	Mouse	Human
Scientific name	*Saccharomyces cerevisiae*	*Chlamydomonas reinhardtii*	*Physcomitrella paten*	*Selaginella moellendorffii*	*Oryza sativa*	*Arabidopsis thaliana*	*Caenorhabditis elegans*	*Drosophila melanogaster*	*Mus musculus*	*Homo sapiens*
Total	30	69	**125**	**122**	**133**	**130**	55	56	53	49
ABCA	0	4	8	6	5	12	7	10	15	12
ABCB	4	8	**20**	**18**	**26**	**30**	21	10	12	11
ABCC	6	12	14	25	17	17	9	12	11	13
ABCD	2	1	6	10	3	2	5	2	4	4
ABCE	1	0	0	0	2	3	1	1	1	1
ABCF	5	0	0	5	6	5	3	3	4	3
ABCG-half	1	9	**23**	**28**	**30**	**28**	9	15	6	5
ABCG-full	9	11	**19**	**24**	**20**	**15**	0	0	0	0
ABCI	2	7	24	6	16	16	0	0	0	0
Undefined	0	17	11	0	8	0	0	3	0	0

Table 2. Numbers of Each Subfamily of ABC Transporters and the Total Number of ABC Transporters in Different Organisms.

Note the specific proliferation of ABCB and ABCG subfamily members in land plants (moss, spikemoss, rice, and *Arabidopsis*). The numbers of ABC transporters in the table are based on the following publications: yeast (Paumi et al., 2009; Brohee et al., 2010; Prasad and Goffeau, 2012; Piecuch and Oblak, 2014), *Chlamydomonas* (Merchant et al., 2007), moss (Lang et al., 2008; Buda et al., 2013), *Selaginella* (Banks et al., 2011), rice (Nguyen et al., 2014), *Arabidopsis* (Verrier et al., 2008; Kang et al., 2011), *C. elegans* (Sheps et al., 2004), fruit fly (Dean and Annilo, 2005), mouse (Mutch et al., 2004), and human (Almen et al., 2009; Vasiliou et al., 2009). In addition, we consulted UniProt (http://www.uniprot.org/uniprot/) for *Chlamydomonas*, moss, *Selaginella*, and human genes and TAIR10 (https://www.arabidopsis.org/) for *Arabidopsis*. The numbers of transporter subfamily members were obtained from the membrane transporter database (Ren et al., 2007), and then adjusted according to the UniProt database and recent studies in the literature. Numbers in bold indicate categories particularly enriched in land plants.

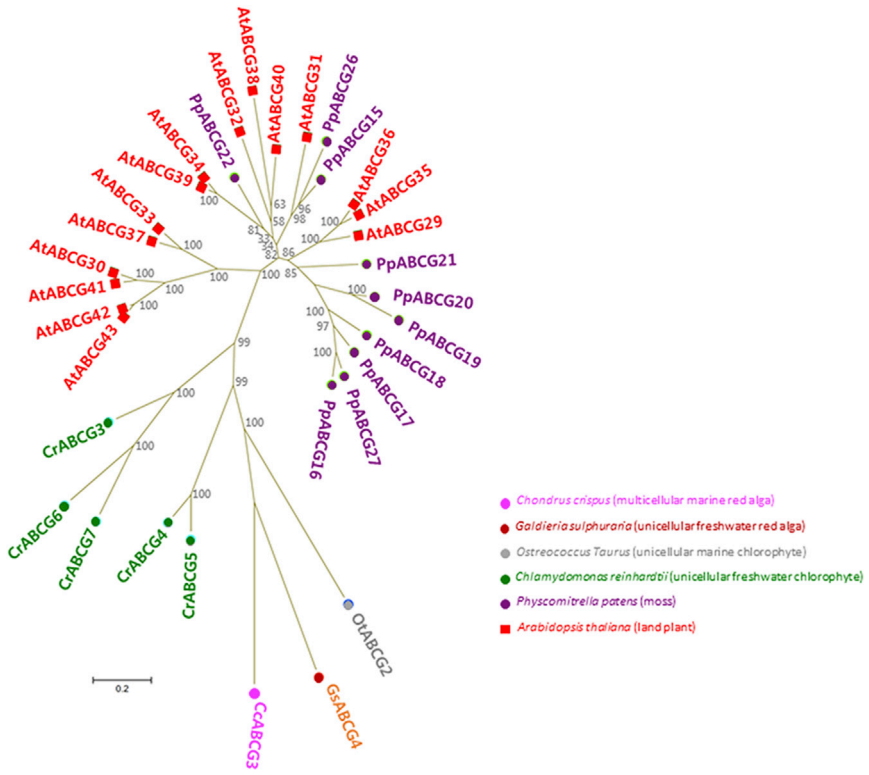

Figure 2. Phylogenetic Relationship of AtABCG40 Homolog (PDR-type ABCG Full-Length Transporter) Sequences from Green Land Plants with Two Red Algal Outgroup Taxa.

This tree demonstrates the expansion of ABCG40 isoforms during the transition from a marine to land environment. Abbreviations and accession numbers (in parentheses) for each sequence: Rhodophyta (marine red algae): *Chondrus crispus*, CcABCG3 (XP_005714333.1); *Galdieria sulphuraria*, GsABCG4 (XP_005706057.1). Chlorophyta (green algae): *Ostreococcus taurus*, OtABCG2 (CEF98550.1), *Chlamydomonas reinhardtii*, CrABCG3 (XP_001697890.1), CrABCG4 (XP_001692307.1), CrABCG5 (XP_001692206.1), CrABCG6 (XP_001694603.1), CrABCG7 (XP_001697137.1). Embryophyta (land plants): *Physcomitrella patens*, PpABCG16 (XP_001764987.1), PpABCG17 (XP_001754895.1), PpABCG18 (XP_001759317.1), PpABCG19 (XP_001773997.1), PpABCG20 (XP_001763163.1), PpABCG21 (XP_001774693.1), PpABCG22 (XP_001783751.1), PpABCG26 (XP_001785866.1), PpABCG27 (XP_001754894.1); *Arabidopsis thaliana*, AtABCG29 (AB29G_ARATH), AtABCG30 (AB30G_ARATH), AtABCG31 (AB31G_ARATH), AtABCG32 (AB32G_ARATH), AtABCG33 (AB33G_ARATH), AtABCG34 (AB34G_ARATH), AtABCG35 (AB35G_ARATH), AtABCG36 (AB36G_ARATH), AtABCG37 (AB37G_ARATH), AtABCG38 (AB38G_ARATH), AtABCG39 (AB39G_ARATH), AtABCG40 (AB40G_ARATH), AtABCG41 (AB41G_ARATH), AtABCG42 (AB42G_ARATH), AtABCG43 (AB43G_ARATH). The tree with the highest log likelihood (−29236.8541) inferred from a maximum likelihood analysis is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers at the nodes reflect the percentage of 1000 replicate bootstrap trees, which resolve the clade at the endpoints of that branch. The analysis involved 33 amino acid sequences. In the alignment, all positions containing gaps and missing data were eliminated. There were a total of 744 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). Scale bar, 0.2 amino acid substitutions per site.

reduced content of wax components such as alkanes, ketones, and primary and secondary alcohols. Although ABCG12 is expressed in the epidermal cells of stems, leaves, siliques, flowers, and roots, cuticle defects were apparent only in *abcg12* stems, suggesting the existence of redundant wax transporters in other tissues. ABCG11 is co-expressed with ABCG12 in the stem epidermis (Suh et al., 2005; Bird et al., 2007) and the corresponding knockout, *abcg11*, exhibited a more drastic phenotype, including post-genital organ fusion, morphologically altered cuticle, cytoplasmic inclusions, and stunted growth, and its wax and cutin amounts were strongly reduced in the stem (Bird et al., 2007; Luo et al., 2007; Panikashvili et al., 2007; Ukitsu et al., 2007; Panikashvili et al., 2010). The strong organ fusion phenotype of this mutant indicates the importance of lubrication by the hydrophobic materials carried by the transporter in organ separation. Later studies revealed that ABCG11 and ABCG12 act in the same pathway in cuticular wax export and that ABCG11 and ABCG12 form a heterodimer (McFarlane et al., 2010). While the transport activity of ABCG12 strictly depends on its interaction with ABCG11, ABCG11 can form functional homodimers. ABCG13 belongs to the same clade as ABCG12 and is required for secretion of cutin monomers in flowers, particularly petals (Sanchez-Fernandez et al., 2001). The *abcg13* knockout displayed floral organ fusion and abnormal epidermal cell structure in petals (Panikashvili et al., 2011). Almost all types of cutin monomers were substantially less abundant in *abcg13* flowers, while the amount of waxes did not change. The substrate specificity of ABCG12 and ABCG13 seems to be narrower than that of ABCG11, which transports a broad range of chemicals required for both cutin and wax deposition (Pighin et al., 2004; McFarlane et al., 2010).

ABCG32/PDR4/PEC1 is a pleiotropic drug resistance (PDR)-type full-size ABCG transporter. The corresponding mutant exhibited increased cuticle permeability in leaves and petals with no other obvious aberrant phenotype (Bessire et al., 2011). ABCG32 is polarly localized at the plasma membrane toward the site where the cuticle is produced, which is in line with its function in secretion to the surface. In the *abcg32* mutant, the amount of typical aliphatic components of cutin was reduced, particularly in the petals, and lipidic inclusions were present in the epidermal cells of the petals. Moreover, this mutant has a less dense cuticular layer than the wild type, and structural changes in the nano-ridge in petals. These results suggest that ABCG32 exports cutin precursors from the epidermal cells in petals.

Formation of sexual reproductive organs was a key event in land adaptation (Wallace et al., 2011). Vascular plants form spores or pollen for sexual reproduction. A protective surface structure enabled these reproductive cells to withstand the dry and changing environment until fertilization (Wellman, 2004). In

plants, spores and pollen possess a unique outer wall structure in the form of a multi-layered wall and coat. In *Arabidopsis*, the pollen wall layers consist of the inner intine and outer exine layers. The exine layer can be further divided into the nexine and sexine layers. The exine is composed of sporopollenin, which is synthesized in the surrounding tapetal cell layer, and transported to the developing microspore surface. The pollen coat, the outermost layer of pollen, also consists of materials synthesized in the tapetum. Many ABC transporters have been reported to have important functions in sporopollenin deposition. AtABCG26 transports polyketides, a major component of sporopollenin, from the tapetum to the microspore (Quilichini et al., 2014). Loss of AtABCG26 results in a severely defective exine layer, and degeneration of most of the microspores during development. Rice OsABCG15 is a putative ortholog of AtABCG26, and *osabcg15* exhibits a defective exine structure, similarly to *atabcg26* (Niu et al., 2013; Wu et al., 2014). However, *osabcg15* also has defects in the anther cuticle, while such defect was not observed in *atabcg26* (Wu et al., 2014). These results suggest that AtABCG26 and OsABCG15 are conserved with respect to their functions in male organ development, but that their ranges of substrate specificity differ.

A double knockout mutant of AtABCG1 and AtABCG16 has defects in pollen nexine formation (Yadav et al., 2014), but the identity of the substrates of AtABCG1 and AtABCG16 remains to be revealed. Suberin-like materials may be their substrate, since they belong to a clade of genes that is involved in root suberin deposition. AtABCG9 and AtABCG31 play important roles in pollen coat maturation (Choi et al., 2014). Mature pollen of *atabcg9 atabcg31* was as viable as that of the wild type under normal growth conditions, but viability was highly reduced under drought or cold stress. Pollen coat maturation was incomplete in the *atabcg9 atabcg31* double knockout mutant. Chemical analysis revealed that steryl glycoside content was highly reduced in *atabcg9 atabcg31* pollen, and this was suggested to be responsible for the defects in the pollen coat, since a steryl glycoside synthesis mutant exhibited a similar defect in pollen coat.

Little is known about the function of ABC transporters in spore wall formation in early terrestrial plants. In the moss *Physcomitrella patens*, the knockout mutant of PpABCG7 exhibits defective cuticular wax accumulation, reduced desiccation tolerance, and altered spore exine structure (Buda et al., 2013). PpABCG7 is a putative ortholog of cuticle precursor transporters in *Arabidopsis*. Thus, ABC transporters involved in surface formation seem to have been conserved during evolution.

Transport of Defense-Related Chemicals

To withstand and ward off pathogens, effective defense systems are essential for all organisms. Plants have developed diverse defense systems, many of which rely on chemicals called secondary metabolites, including terpenoids, alkaloids, and phenolics. However, accumulation of these chemicals within the cell can be toxic to plants themselves. Plants have evolved two strategies to cope with the potential toxicity of these compounds; they are either exported to the cell surface or non-toxic precursors are stored within the vacuole. In the latter case, the compounds are hydrolyzed and become toxic only when cells are destroyed by pathogens. Many studies have shown that ABC transporters transport secondary metabolites.

The ABCG transporters NpPDR1/NpABC1 and NtPDR1 are induced by sclareol and jasmonate. These transporters are highly expressed in the leaf epidermis cells and trichomes of tobacco, and transport sclareol, which is a diterpenoid secreted by *Nicotiana* species (Jasiński et al., 2001; Stukkens et al., 2005; Crouzet et al., 2013). Similarly, SpTUR2, a homolog of *Nicotiana* PDRs in the aquatic plant *Spirodela polyrhiza*, confers tolerance to sclareol when expressed in *Arabidopsis* (Van Den Brûle et al., 2002). AtABCG40 is suggested to have a role in pathogen defense, since an *atabcg40/atpdr12* plant exhibits sensitivity to sclareol (Campbell et al., 2003). Although sclareol is not likely the substrate of SpTUR2 and AtPDR12, because it is not synthesized in *S. polyrhiza* or *Arabidopsis*, the results suggest that these transporters transport similar chemicals produced by the corresponding plants in response to pathogen attack.

Berberine, a yellow benzylisoquinoline alkaloid, is a potent antimicrobial compound that inhibits gram-negative and gram-positive bacteria, and has antifungal activity. Berberine accumulates in large amounts in *Coptis japonica* rhizomes, protecting them from pathogens. CjABCB1/CjMDR1 is localized at the plasma membrane, expressed preferentially in the xylem tissue of rhizomes, and catalyzes the import of berberine, thus playing a role in the translocation of berberine from the root to the rhizome (Yazaki et al., 2001; Shitan et al., 2003). CjABCB2 was reported to have similar functions to CjABCB1 (Shitan et al., 2003).

Vinblastine and vincristine are highly toxic compounds produced by *Catharanthus roseus* that are used in cancer therapy. Their synthesis is complex and occurs in different cell types, necessitating transport of intermediates between the cells involved. Interestingly, while vinblastine and vincristine are localized in special leaf cells of *C. roseus*, a major metabolite of vincristine, catharanthine, is transported mainly to the cell surface. Yu and De Luca (2013) showed that CrTPT2, a full-size ABCG transporter of *C. roseus*, is localized to the plasma membrane, and functions as an efflux transporter that transports catharanthine to the cell surface.

Arabidopsis PDR8/ABCG36/PEN3 blocks the penetration of non-host fungal pathogens (Stein et al., 2006). AtPDR8 is localized at the plasma membrane, recruited to the infection site (Underwood and Somerville, 2013), and involved in glucosinolate-dependent pathogen defense at the contact site (Clay et al., 2009). The identity of the chemical secreted by this transporter, which provides effective defense against extracellular pathogens, is unknown. Recently, Lu et al. (2015) proposed that the precursor(s) of 4OGlcI3F (4-75 *O*-β-D-glucosyl-indol-3-yl formamide) is the substrate of AtPDR8.

The phenolics, including flavonoids, anthocyanins, and proanthocyanidins, are another class of secondary metabolites produced by plants that exhibit diverse roles, such as protection against pathogen attack, UV screening, signaling, or attracting pollinators (Freeman and Beattie, 2008). Flavonoids, which have antioxidant activity, are the largest class of phenolics (Treutter, 2006). Anthocyanins function as pollinator attractants

and UV screens, while proanthocyanidins function as defense molecules that repel insects. While proanthocyanidins are transported exclusively by MATE-type transporters (Zhao, 2015), anthocyanins are transported by both MATE-type and ABC transporters. A genetic screen of *Zea mays* (maize) suggested that two ABCC transporters, ZmMRP3 and ZmMRP4, are required for anthocyanin accumulation in maize kernels (Goodman et al., 2004). However, no direct proof for the transport activity was provided. In a later study with a homolog of the maize transporter from *Vitis vinifera* (grapevine), VvABCC1, anthocyanin transport activity was demonstrated by heterologous expression in yeast (Francisco et al., 2013). Isoflavonoids are a class of phytoalexins with antibiotic and antifungal properties. They are produced in response to pathogen attack and are often pathogen specific. The isoflavonoid medicarpin is produced by Medicago species, and MtABCG10, a close homolog of NtPDR1, transports medicarpin in response to pathogen attack (Banasiak et al., 2013).

Triticum aestivum (wheat) is subject to infection by diverse fungal pathogens. *Lr34* is one of the few durable resistant genes identified to confer resistance to multiple fungal pathogens in wheat. Positional cloning revealed that this gene encodes a PDR-type ABCG transporter (Krattinger et al., 2009). However, even 6 years after its discovery, the substrate transported by Lr34 is unknown. In addition to their role in pathogen defense, ABC transporters also provide protection against insects, probably by transporting deterring compounds. NtABCG5/NtPDR5 is induced by insect herbivory and the absence of this transporter decreases resistance to insects (Bienert et al., 2012). In a proteomic analysis of *Mentha spicata* (spearmint) trichomes, 11 ABC transporters were identified, supporting the role of ABC transporters in monoterpenoid secretion and thus in protection against herbivores (Champagne and Boutry, 2013).

Interestingly, many full-size ABCG/PDR-type ABC transporters are induced by biotic stresses (Kang et al., 2011). For instance, AtABCG40/AtPDR12 is induced by infection with fungal and bacterial pathogens (Campbell et al., 2003); AtABCG16 is induced by bacterial infection and coronatine (Ji et al., 2014); and four ABC transporters (StPDR1 to StPDR4) of potato (*Solanum tuberosum*) are induced by pathogen infection and sclareol (Ruocco et al., 2011). However, induction of ABC transporters by a substrate that is not produced by the plant analyzed provides only hints as to the identity of the *in vivo* substrate for the transporter. These results should thus be regarded as preliminary, and additional studies of ABC transporters' endogenous substrates are merited. Indeed, much effort is needed to identify the exact substrates of the many ABCG transporters that contribute to plant's defense responses.

Transport of Cytokinins, a Signal Sent from the Root to the Shoot

Terrestrial plants have evolved two specialized structures, roots and shoots, to obtain water and minerals from the soil and to conduct photosynthesis in the air, respectively. The differentiation of the root and shoot required that a communication system be established between the two structures to coordinate their physiological and developmental processes. Cytokinins are signaling molecules that facilitate communication between the below- and above-ground structures. When conditions are favorable for growth, the root synthesizes *t*Z-type cytokinins, which are translocated to the shoot through the xylem to stimulate shoot growth (Kiba et al., 2013). Shoot-derived iP-type cytokinins are transmitted to the root via phloem and regulate vascular differentiation in the root meristem (Bishopp et al., 2011).

Recently, it was reported that the AtABCG14 transporter is essential for the root-to-shoot translocation of cytokinins (Ko et al., 2014; Zhang et al., 2014b). The transporter is localized at the plasma membrane and primarily expressed in the root pericycle, phloem, and procambial cells, in which cytokinin biosynthesis genes, such as adenosine phosphate-isopentenyl transferases and *CYP735A2*, are highly expressed. The expression of *AtABCG14* in cells that synthesize cytokinins and its localization at the plasma membrane support the notion that AtABCG14 is an efflux transporter that translocates cytokinin to the shoot. The *atabcg14* mutant exhibits severe shoot growth retardation (Figure 3), which is recovered by exogenous *t*Z treatment, suggesting that the shoot growth defect is due to a deficiency of *t*Z-type cytokinins. Indeed, the content of *t*Z-type cytokinins is dramatically reduced in the shoots of the *atabcg14* mutant, but elevated in the mutant roots. These results suggest that the *atabcg14* mutant cannot load *t*Z-type cytokinins into the xylem and, consequently, that *t*Z-type cytokinins remain in the root. To test this hypothesis, cytokinin levels in the xylem sap were quantified. The endogenous levels of *t*Z-type cytokinins in the xylem sap of the *atabcg14* mutant were dramatically reduced compared with those of the wild type (Ko et al., 2014), implying that xylem loading of the hormone for long-distance transport is defective in the mutant. Consistently, translocation assays using exogenous radiolabeled *t*Z showed that acropetal transport of *t*Z is substantially reduced in the *atabcg14* mutant compared with the wild type. In addition, the importance of AtABCG14-mediated cytokinin translocation for shoot growth was confirmed by grafting experiments; the growth defects of the *atabcg14* mutant shoot were rescued by grafting to the wild-type root, whereas growth was retarded, as in the *atabcg14* mutant, when the wild-type shoot was grafted to the *atabcg14* mutant root. Taken together, these studies clarified that AtABCG14 is essential for long-distance communication from the root to the shoot by mediating xylem loading of *t*Z-type cytokinins in roots to stimulate shoot growth (Ko et al., 2014; Zhang et al., 2014b).

Transport of Abscisic Acid that Mediates Stress Tolerance and Inhibition of Seed Germination

Drought is the most damaging abiotic stress affecting plant productivity. ABA levels increase rapidly upon exposure to drought and lead to stomatal closure and induction of stress-related genes, which allow the plant to withstand drought (Lee and Luan, 2012). Recent studies that analyzed the expression of ABA-related genes, endogenous ABA levels, and movement of radiolabeled ABA raised the possibility that ABA is transported from cell to cell and tissue to tissue (Everant-Bourbouloux, 1982; Nambara and Marion-Poll, 2005; Endo et al., 2008;

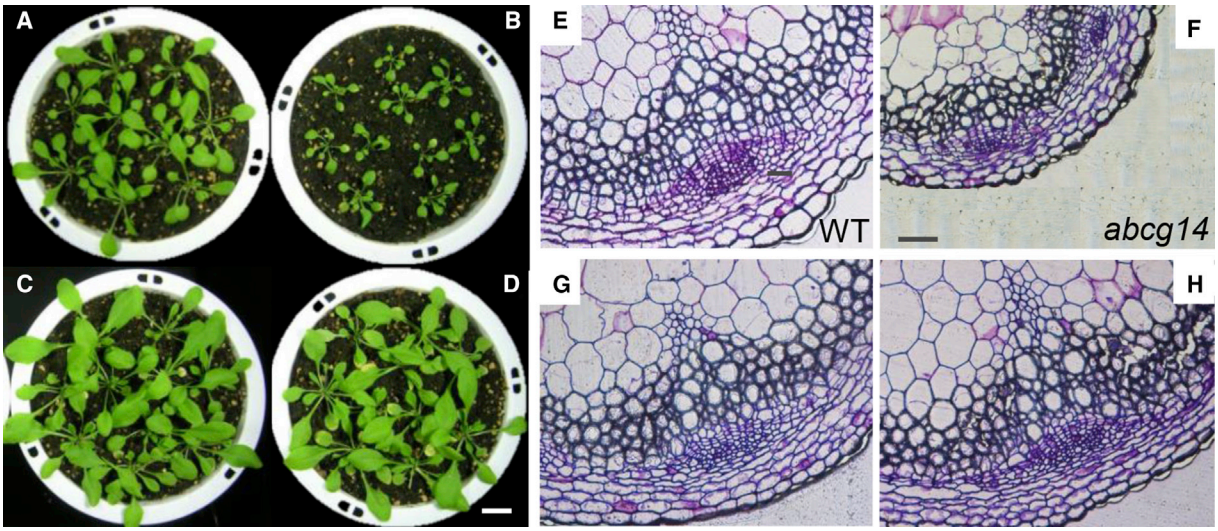

Figure 3. *atabcg14* Knockout Plants Exhibit Shoot Growth Retardation.
AtABCG14 mediates translocation of cytokinin from the root, where it is synthesized, to the shoot, where it activates cell division and growth. *atabcg14* knockout plants are small **(B)** in comparison to the wild type **(A)** and the two complementation lines **(C and D)**, with a reduced number of xylem and phloem cells (compare **E** and **F**). The complementation lines **(C, D, G, and H)** recover the shoot growth defects of the *atabcg14* mutant (adapted from Ko et al., 2014). Scale bars, 4 cm **(A–D)** and 80 μm **(E–H)**. WT, wild type.

Ikegami et al., 2009; Kanno et al., 2010; Seo and Koshiba, 2011; Kuromori et al., 2014). Since the pK_a of ABA is 4.7, cytosolic ABA is present in its de-protonated, negatively charged form (cytosolic pH ~7). Therefore, export of cytosolic ABA to the apoplast requires an export machinery. It has been shown that, under drought stress, the apoplastic pH increases (Wilkinson and Davies, 1997). At high pH, the ratio of negatively charged, less permeable ABA to protonated, uncharged ABA strongly increases and most ABA cannot freely enter cells. Consequently, ABA diffusion into neighboring cells is reduced, and ABA, instead, undergoes long-distance transport. The recent identification of many ABA transporters at the plasma membrane of diverse tissues supports the notion that ABA is transported throughout the plant (Kang et al., 2010; Kuromori et al., 2010; Kanno et al., 2012; Zhang et al., 2014a; Kang et al., 2015).

Genetic and functional screens have revealed four ABC transporter family members implicated in ABA transport in *Arabidopsis*. AtABCG25 exhibits ABA-efflux activity, and *atabcg25* mutants displayed several ABA-related phenotypes, such as impaired stomatal movement and germination (Kuromori et al., 2010). *AtABCG25* is expressed mainly in vascular tissues, suggesting that AtABCG25 plays an important role in exporting ABA from the vasculature, which is one of the major ABA production sites. By contrast, AtABCG40 is an ABA uptake transporter. Overexpression of AtABCG40 leads to increased ABA accumulation in tobacco and yeast cells, whereas ABA uptake into *atabcg40* mutant cells was reduced. Furthermore, *atabcg40* mutant plants exhibited a defect in stomatal closure, resulting in enhanced water loss (Kang et al., 2010). More recently, two additional ABC members, AtABCG30 and AtABCG31, have been revealed as an ABA importer and an exporter, respectively. In *Arabidopsis* seeds, endosperm-expressed AtABCG31 and embryo-expressed AtABCG30 were found to cooperate to suppress germination of imbibed seeds (Kang et al., 2015). This work also confirmed the previously reported functions of ABCG25 (Kuromori et al., 2010) and AtABCG40 (Kang et al., 2010) in seed germination. Thus, at least four ABC transporters suppress embryonic growth in the seed, until conditions are favorable for germination. Undoubtedly, these transporters contributed to the success of terrestrial plants in colonizing broad areas of land, since they allowed seeds to travel great distances from the mother plant and colonize new territories. It is surprising that all knockouts of these transporters exhibited aberrant phenotypes, albeit some of them are marginal, suggesting that their functions are not completely redundant. Additional ABA transporters not belonging to the ABC family have also been discovered (Merilo et al., 2015) and additional ABA transporters belonging to the ABC, NRT, or MATE families, or even to some other transporter family, may still be identified.

Transport of Auxin for Growth and Tropism

Once a seed germinates, the plant cannot move, and has to adapt to the ever-changing, heterogeneous environmental conditions surrounding it. Plants have developed a growth response named tropism to cope with directional differences in environmental conditions. Tropisms are the result of differential cellular growth and development in response to the vectorial input of light (phototropism), gravity (gravitropism), water (hydrotropism), or physical contact (thigmotropism). The directional growth of plants is governed by the spatiotemporal asymmetric distribution of the growth hormone, auxin, and the asymmetric auxin distribution depends on auxin transporters.

Indole-3-acetic acid (IAA) is the most abundant endogenous auxin. In addition to PINs and AUX/LAX-type transporters (see recent reviews for AUX/LAX and PIN protein families), many members of the ABCB transporter family have been shown to be involved in IAA transport, based on their loss-of-function mutant phenotypes and results obtained with biochemical transport assays (Cho and Cho, 2013). For instance, AtABCB1 and

AtABCB19 were shown to have IAA efflux transport activity when expressed in heterologous systems (Geisler et al., 2005; Yang and Murphy, 2009). Their loss-of-function mutants displayed pleiotropic auxin-related phenotypes, and *atabcb1 atabcb19* plants exhibited dwarfism (Geisler et al., 2005; Lewis et al., 2009; Cecchetti et al., 2015). AtABCB4 exhibited concentration-dependent influx/efflux transporter activity (Terasaka et al., 2005; Yang and Murphy, 2009), and loss-of-function mutants of AtABCB4 had an abnormal gravitropism response, aberrant lateral root formation, and enhanced root hair elongation (Terasaka et al., 2005; Cho et al., 2007). In support of their roles in polar auxin transport, mutations in these *ABCB* genes alter auxin transport; *atabcb1*, *atabcb4,* and *atabcb19* plants are defective in basipetal auxin transport in roots (Terasaka et al., 2005; Blakeslee et al., 2007) and *atabcb1* and *atabcb19* leaf protoplasts are reduced in net auxin efflux (Geisler et al., 2005). AtABCB21 transports IAA when expressed in yeast cells (Kamimoto et al., 2012), but no *atabcb21* knockout mutant has been characterized. Thus, the *in vivo* auxin transport activity of AtABCB21 and its role in mediating auxin-dependent signals remain to be established. More recently, a study identified two more auxin transporter candidates (Kaneda et al., 2011): AtABCB14 and AtABCB15 are expressed during lignification of *Arabidopsis* stems, and their loss-of-function mutations result in a reduction of polar auxin transport in the inflorescence stem. However, biochemical analyses are necessary to determine whether they indeed transport auxin or if they affect auxin transport indirectly. AtABCB14 was previously reported to transport malate when expressed in *Escherichia coli* or in HeLa cells (Lee et al., 2008). The malate transport activity of ABCB14 was suggested to contribute to the stomatal response to CO_2. So far, it is an open question whether ABCB14 indeed transports auxin in addition to malate, or whether the auxin-related phenotypes of *abcb14* are indirect effects of the lack of malate transport.

Polar auxin transport, which is the basis of the directional growth of plants, relies largely on the polar localization of PIN auxin transporters (PIN1 and PIN2). In contrast to PIN1 and PIN2, ABCB1, ABCB4, and ABCB19 predominantly exhibit nonpolar and stable plasma membrane localization in root apexes (Geisler et al., 2005; Blakeslee et al., 2007). Therefore, these ABCB proteins were suggested to function in non-directional auxin export. However, experimental results indicate that they contribute to polar auxin transport. The *atabcg4* knockout mutant exhibits reduced basipetal auxin transport in roots (Terasaka et al., 2005; Yang and Murphy, 2009), and ABCB19 stabilizes PIN1 in the plasma membrane (Blakeslee et al., 2007). In addition, AtABCB1 and AtABCB4 are localized non-symmetrically at the plasma membrane in mature root cells (Geisler et al., 2005; Blakeslee et al., 2007). Thus, the function of ABCB proteins in polar auxin transport is diverse and complex; they maintain the auxin gradient established by the PINs by exporting auxin, they stabilize PINs, and, in some cases, function as polar auxin transporters themselves (Geisler et al., 2005; Blakeslee et al., 2007).

Indole 3-butyric acid (IBA) is a natural auxin that functions as an important precursor of IAA (Strader and Bartel, 2011). The knockout mutants of two ABCG transporters, AtABCG37 and AtABCG36, are hypersensitive to auxinic compounds, and AtABCG37 and AtABCG36 are reported to transport a range of auxinic compounds, including IBA, but not free IAA (Strader and Bartel, 2009; Ruzicka et al., 2010). AtABCG36 and AtABCG37 specifically localize to the outermost plasma membrane in roots, and thus were suggested to export IBA to the rhizosphere (Strader and Bartel, 2009; Ruzicka et al., 2010). However, IBA may not be a primary transport substrate for ABCG36 or 37, since these proteins were reported to transport other substrates, such as terpenoids and phenolics, and to be involved in many other physiological phenomena, such as iron nutrition (Ito and Gray, 2006; Fourcroy et al., 2014; Lu et al., 2015) and tolerance to abiotic stress (Kim et al., 2007, 2010). The physiological relevance of the IBA transport activities of these transporters remains to be elucidated.

In nature, numerous precursors and structurally modified forms of auxin exist. Considering the importance of auxin homeostasis, plants most likely use many different types of transporters to transport these compounds into and out of the cells. Therefore, we predict that additional members of the ABC family and other transporters transport or modulate the transport of auxin and its derivatives.

Transport of Strigolactone for Interactions with Microorganisms and Control of Shoot Branching

Strigolactones were identified as growth stimulants for the parasitic weed *Striga*, and for many years the physiological role of strigolactones in plants was the subject of speculation (Seto and Yamaguchi, 2014; Waldie et al., 2014). Strigolactones are carotenoid-derived sesquiterpenes synthesized by two carotenoid cleavage dioxygenases and a cytochrome P450 (Seto and Yamaguchi, 2014). Nearly 40 years after the first chemical description of strigolactones, Akiyama et al. (2005) finally discovered their role in inducing hyphal branching of mycorrhizal fungi, a process that greatly facilitates mycorrhization. Three years later, two laboratories found that the long-sought factor that together with auxin inhibits lateral shoot branching was a strigolactone (Gomez-Roldan et al., 2008; Umehara et al., 2008). Based on the finding that full-size ABCGs are localized at the plasma membrane and the suggestion that they excrete diterpenes (Jasiński et al., 2001), Kretzschmar et al. (2012) postulated that an ABC transporter of this class also mediates strigolactone excretion. They identified a candidate gene, *PaPDR1*, which was induced both by strigolactones and by limited Pi availability, conditions that favor mycorrhization (Kretzschmar et al., 2012), and showed that PaPDR1 is localized in the root tip and hypodermal passage cells, which constitute entry points for mycorrhizal fungi. The latter result was confirmed in a subsequent study (Sasse et al., 2015). Furthermore Kretzschmar et al. (2012) showed that mycorrhization of mutant plants was markedly delayed due to a strong reduction in strigolactone excretion. The observation that transgenic *Arabidopsis* plants expressing PaPDR1 had increased resistance to toxic doses of artificial strigolactone GR24 due to their ability to excrete this compound more efficiently suggests that PaPDR1 indeed acts as a strigolactone transporter. Later studies (Sasse et al., 2015) showed that PaPDR1 exhibits a dual polar localization. The transporter is polarly localized in root tip cells, which synthesize strigolactones in the apex, and promotes transport

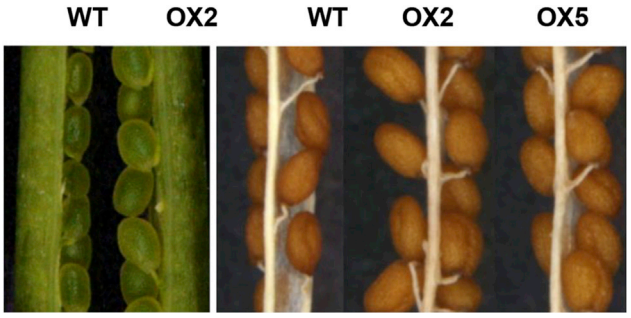

Figure 4. AtABCA9-Overexpressing Plants (OX2 and OX5) Produced Larger Seeds than the Wild Type.
The overexpressors also contained increased amounts of neutral lipids (triacylglycerol), which can readily be converted to fuel (from Kim et al., PNAS, USA, 2013). Thus, AtABCA9 expression can be manipulated to increase biodiesel production from plant seeds. WT, wild type.

toward the shoot. In hypodermal passage cells, PaPDR1 is laterally localized, which is in agreement with its function as a strigolactone secretion system. However, how exactly strigolactones are transported from the root tip to the shoot is so far unknown.

PaPDR1 appears also to play an important role in strigolactone transport in the aerial parts of *Petunia hybrida* (petunia). Petunia and tobacco strigolactone transporter mutant plants branch more frequently than the corresponding wild type (Kretzschmar et al., 2012; Xie et al., 2015). Furthermore, in both cases, the transporters are expressed in the vasculature and strongly in the nodes of lateral branches, sites that likely deliver strigolactones to the lateral buds. Strigolactones are not only produced in roots but also in shoots. Previous grafting experiments indicated that root- and shoot-derived strigolactones have the same effect on shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008). However, it would be interesting to re-examine whether root- and shoot-derived strigolactones are delivered to the same or different cells, and whether they exhibit similar or different functions, since our knowledge of strigolactones has increased tremendously recently.

Transport of Intermediates and Storage Compounds of the Complex Biochemical Network Inside the Cell

Synthesis of metabolites and storage compounds often requires one or more transport steps through different membranes. Five ABC transporters that facilitate the transport of lipidic metabolic intermediates between cell compartments have been isolated to date: AtABCD1/PXA1, AtABCI14/TGD1, AtABCI15/TGD2, AtABCI13/TGD3, and AtABCA9.

The peroxisomal transporter AtABCD1/PXA1 was identified in three independent screens for auxin IBA-resistant mutants, 2,4-dichlorophenoxybutyric acid (2,4-DB)-resistant mutants, and impaired germination mutants, and was named PEROXISOMAL ABC TRANSPORTER 1 (PXA1; Zolman et al., 2001), PEROXISOME DEFICIENT 3 (PED3; Hayashi et al., 2002), and COMATOSE (CTS; Russell et al., 2000; Footitt et al., 2002), respectively. Peroxisomes are the site of β-oxidation, through which fatty acids are degraded to supply the energy required for germination of oil-storing seeds. PXA1 is necessary for peroxisomal import of fatty acids/acyl-CoA and IBA for β-oxidation. The sequential activities of PXA1 and peroxisomal long-chain acyl-CoA synthetases (LACS6/LACS7) provide acyl-CoA for β-oxidation in plant peroxisomes (Fulda et al., 2004). The basal ATPase activity of PXA1 was stimulated by fatty acyl-CoAs, rather than by free fatty acids (Nyathi et al., 2010), suggesting that fatty acyl-CoAs are the substrates of the transporter. Thus, the involvement of LACS6/LACS7 in the β-oxidation process was initially puzzling, but was resolved when PXA1 was shown to exhibit thioesterase activity, which is stimulated by ATP and is essential for fatty acid degradation (Lousa et al., 2013). Taken together, PXA1 seems to provide fatty acids and CoA using its own thioesterase and transporter activity, and subsequently, LACS6/LACS7 synthesize acyl-CoA again in the plant peroxisome. Recently, PXA1 was found to interact with and be activated by COMPARATIVE GENE IDENTIFICATION-58 (CGI-58; Park et al., 2013), which regulates TAG metabolism and lipid signaling in mammals and in non-lipid-storing cell types of plants (James et al., 2010). CGI1 positively regulates PXA1 functions in the turnover of polyunsaturated free fatty acids and the metabolism of auxin and jasmonic acid precursors. Thus, PXA1 is a transporter that has an impact on energy metabolism as well as in signaling.

Three ABC transporters important for membrane lipid metabolism were identified in a screen for galactoglycerolipid trigalactosyldiacylglycerol (TGDG) over-accumulation mutants: AtABCI14/TGD1 (Xu et al., 2003), AtABCI15/TGD2 (Lu and Benning, 2009), and AtABCI13/TGD3 (Awai et al., 2006). These proteins mediate ER-to-chloroplast lipid trafficking by forming a bacterial-type ABC transporter complex at the chloroplast inner envelope membrane (Roston et al., 2012). TGD1, TGD2, and TGD3 function as the permease, the substrate-binding protein, and the ATPase, respectively (Xu et al., 2003; Awai et al., 2006; Lu et al., 2007; Lu and Benning, 2009; Roston et al., 2012). TGD1, TGD2, and TGD3 also interact with a small glycine-rich protein, TGD5, at the plastidial inner envelope (Fan et al., 2015). TGD5 additionally binds to TGD4, a transmembrane lipid transfer protein localized in the outer envelope (Xu et al., 2008), thus connecting the inner and outer envelope membranes of the plastid. The whole TGD protein complex imports ER-derived phosphatidic acid (PA) to the inside of the inner envelope membrane, where PA phosphatase produces the diacylglycerol (DAG) required for galactolipid biosynthesis.

Triacylglycerol (TAG) is synthesized in the ER, using fatty acid building blocks made in the chloroplast. However, the mode of fatty acid transport from the chloroplast to the ER was unknown until recently. An ER-localized ABC transporter AtABCA9 was reported to be important for normal accumulation of TAG in developing seeds (Kim et al., 2013). In the *atabca9* null mutant, seed TAG content is reduced by 35% and TAG produced from exogenously fed radiolabeled acyl-CoA or free fatty acids is reduced to about half that of the wild type. Furthermore, overexpression of this transporter increased seed size (Figure 4) and TAG deposition by up to 40%. Thus, AtABCA9 supplies fatty acid substrates for TAG biosynthesis at the ER during the seed-filling stage.

In seeds, phosphate is stored mainly in the form of phytate (inositolhexa*kis*phosphate). Although it was already established

in 2001 that AtABCC5 functions as an ABC transporter that affects stomatal movement (Gaedeke et al., 2001; Klein et al., 2003), phytate was revealed as the substrate of AtABCC5 only in 2007, when a screen for low-phytate crops (Shi et al., 2007) revealed that a maize line defective in a close homolog of AtABCC5 produced low-phytate seeds. Subsequent biochemical analysis of *atabcc5* plants and heterologous expression of *AtABCC5* in yeast cells provided biochemical evidence that AtABCC5 and its close homologs indeed act as high-affinity phytate transporters (Nagy et al., 2009). The observation that the absence of this transporter has such a strong impact on guard cell function is also evidence that inositolhexa*kis*phosphate is indeed an important second messenger in plants.

Transport of Toxic Compounds for Detoxification

Xenobiotics exist in our environment either naturally in soils or due to anthropogenic activities. As sessile organisms, plants have had to adapt especially well to toxic compounds in the environment. Vacuolar sequestration is a final detoxification step of potentially toxic chemicals, heavy metals, and metalloids in plants, and ABC transporters have been shown to play a central role in this process. In a search for ABC transporters involved in arsenic (As) detoxification, Song et al. (2010) observed that the double knockout mutant of AtABCC1 and AtABCC2 was extremely sensitive to As (Figure 5A). When expressed in yeast, the two transporters conferred As resistance only in the presence of phytochelatins (PCs), suggesting that the substrate of the transporter is PC-conjugated As. The vacuolar PC2-As transport activity of the *atabcc1 atabcc2* double mutant was only 15% that of the wild type, and a transport assay using vesicles isolated from yeast expressing either AtABCC1 or AtABCC2 showed that these transporters are indeed able to transport PC-As (Song et al., 2010). Further studies showed that the same transporters are also responsible for Cd and Hg detoxification. Cells of the double knockout mutant accumulated Cd in the cytosol, while Cd was localized in the vacuolar lumen of wild-type cells (Park et al., 2012). These results suggest that AtABCC1 and AtABCC2 represent the major vacuolar PC-heavy metal(loid) transporters in *Arabidopsis*. AtABCC3 exhibits a similar function as AtABCC1 and AtABCC2, but probably plays a minor role (Brunetti et al., 2015).

OsABCC1, an ortholog of AtABCC1 and AtABCC2, was suggested to act as a vacuolar PC-As transporter in rice (Song et al., 2014). *OsABCC1* is expressed throughout the rice plant, predominantly in the exodermis and pericycle of roots and in phloem companion cells of node 1, which participates in As uptake and redistribution into different organs, especially grains. OsABCC1 knockout lines were highly sensitive to As(III) and accumulated >10-fold as much As in the grains, while less As was present in node 1, due to a defect in As sequestration into the vacuoles of roots and phloem of node 1. These results suggest that OsABCC1 plays a pivotal role in As resistance of the whole rice plant and in As accumulation in the rice grain. Consumption of As-contaminated rice causes severe diseases in humans, such as skin lesions, digestive problems, respiratory problems, stroke, and cancer. Tens of millions of people in southeast Asia are exposed to this risk, because rice is a staple food in their diet. Developing a rice plant with low-As accumulating grains would greatly alleviate these health problems. A better understanding of the genes participating in vacuolar sequestration of As is necessary to develop such a crop.

Aluminum (Al) is highly toxic when present in the trivalent form, which is generated under acidic soil conditions. Since 50% of the potentially cultivable land on our planet is acidic (Von Uexküll and Mutert, 1995), Al toxicity is a serious problem in agriculture. Screens for factors involved in Al stress tolerance revealed several ABC transporters involved in this process, including AtABCI16/AtALS3 and AtABCI17/AtSTAR1 and two homologous rice transporters, OsSTAR1 and OsSTAR2. When OsSTAR1 and OsSTAR2 are expressed together, they form a complex that transports UDP glucose (Huang et al., 2009). This may alter the cell wall composition, which in turn confers Al tolerance by inhibiting Al migration into cells. Similarly, AtABCI16 and AtABCI17 form a functional unit, and knockout mutants of AtABCI16 are hypersensitive to Al (Larsen et al., 1997, 2005). Moreover, OsSTAR1 rescued the aluminum-sensitive phenotype of *atabci17*, indicating that the proteins encoded by these genes have similar functions.

Plants also have the capacity to detoxify potentially toxic organic compounds released by pathogens or anthropogenically produced chemicals. The detoxification mechanism and the enzymes involved in this process are highly similar in plants and animals (Kreuz et al., 1996). However, whereas the modified and detoxified compounds are excreted by animals, only a minor fraction are excreted by plants, and plants store most of these compounds in the large, central vacuole. For example, ABC transporters of the C family have been shown to catalyze the vacuolar import of glutathionated and glucuronated xenobiotics (Lu et al., 1997; Klein et al., 1998). In the case of glucosylated compounds, directly energized ABCC-dependent transport has been reported (Dean and Mills, 2004), in addition to the transport driven by secondary active antiporters that exploit the proton gradient between the vacuole and the cytosol (Gaillard et al., 1994; Frangne et al., 2002).

CONCLUSIONS AND FUTURE PERSPECTIVES

It is clear that plant ABC transporters carry diverse molecules important for survival of plants. No other transporter family has been reported to transport such a diversity of chemicals. Different members of the ABC transporter family transport widely different substrates, and, moreover, even a single ABC transporter transports multiple substrates. For example, AtABCG36 transports diverse substrates (Kim et al., 2007; Strader and Bartel, 2009; Ruzicka et al., 2010; Lu et al., 2015). AtABCG40 might also transport multiple substrates in addition to ABA, since it is highly induced upon pathogen infection (Campbell et al., 2003). Such diverse substrate specificities of ABC transporters might have helped plants survive the land environment, which fluctuates more than the aqueous one. This might have been a reason why the plant genome maintained the ABC transporters that were multiplied during evolution.

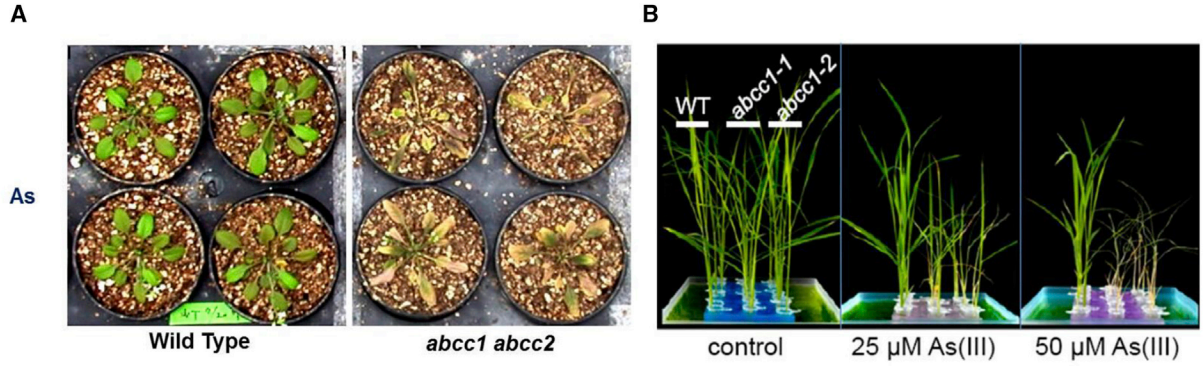

Figure 5. Arsenic Transporters of *Arabidopsis* and Rice.
(A) AtABCC1 and ATABCC2 are arsenic-phytochelatin transporters that confer arsenic resistance in *Arabidopsis*. When watered with As-containing solution, *abcc1 abcc2* double knockout plants (right panel) perished, whereas wild-type plants (left panel) survived (Song et al., 2010).
(B) The ortholog of *Arabidopsis* ABCC1 in rice, OsABCC1, confers arsenic tolerance in rice. The growth of two independent alleles of *abcc1* knockout rice *(abcc1-1, abcc1-2)* is much more impaired than that of the wild type (WT) in arsenic-containing (As(III)) culture medium (Song et al., 2014).

The high level of functional diversification of ABC transporters in green plant lineages during evolution is intriguing from a systems and evolutionary biology perspective. Many questions remain to be answered, such as how did ABC transporters diversify during evolution from microalgae, which clades underwent the highest levels of multiplication, what kind of new substrates did these transporters transport, and how did these transporters help the plant survive on land? Particularly interesting groups are the ABCB and ABCG subfamily members, which are enriched in land plants (Table 2).

Many reports on plant ABC transporters have revealed their mutant phenotypes but not identified their substrates. Four obstacles make substrate identification challenging. First, this group of genes is difficult to handle, due to their large size and the difficulty involved in expressing them in simple heterologous systems such as *E. coli* and budding yeast. Second, conventional flux assays use radioisotope or fluorescently labeled substrates, which are available only for a limited number of compounds. Third, the phenotype of a mutant plant may depend on a complex mix of compounds. Fourth, many ABC transporters transport hydrophobic or complex compounds (such as glutathionated pigments) that are difficult to analyze using conventional methods. Systems biology approaches might help overcome these obstacles, since they are based on large amounts of data, which may facilitate the prediction of potential substrates and domains of ABC transporters important for determining substrate specificity. In addition, large-scale substrate screening combined with advanced analysis techniques for substrate identification might be fruitful.

No plant ABC protein has been crystallized to date. Often the structure of a protein is better conserved than its amino acid sequence, and one can deduce more about common substrates from the structures than from the amino acid sequences. A better understanding on the structures will give rise to more reliable models, which will allow accurate prediction of the substrate recognition pocket and hence potential substrates. Finally, systemic, structural, biochemical, and physiological approaches should be combined in efforts to characterize these transporters.

EXPECTED BENEFITS FROM THE STUDY OF PLANT ABC TRANSPORTERS

Scientists agree that terrestrial plants evolved from aquatic photosynthetic organisms, but they do not agree on the underlying details. New information on a large and ancient gene family, ABC transporters, will provide valuable insight into the evolution of photosynthetic organisms.

Because plant ABC transporters transport molecules that are important for plant growth, such as plant hormones, lipids, metabolites, contaminants, and defense molecules, genetic manipulation of these transporters may form the basis for developing improved crops. For example, knowledge on ABC transporters that transport plant stress and growth hormones can be used to develop crops with improved stress resistance and improved yield and biomass, respectively. Moreover, research on ABC transporters might provide information that can be used to produce biodiesel and other valuable products from microalgae and green plants, since ABC transporters transport economically important compounds, such as lipidic molecules, pigments, and drugs.

As described above, As is sequestrated in the vacuoles of the phloem cells of rice nodes (Song et al., 2014) by an ABC transporter (Figure 5B). Further work using this transporter might provide a strategy for reducing As contamination in rice grains and producing safer food. Furthermore, ABC transporters have important medical applications. Drug resistance is an obstacle in chemotherapy, because cancer cells eliminate drugs by expressing ABC transporters. These transporters also transport cholesterol and other metabolites related to obesity. Because ABC transporters of diverse living organisms are likely to share many properties, including mechanism of transport (Procko et al., 2009), studies of plant ABC transporters may provide insight into ABC transporters in humans. Plants are an ideal material for genetic research, because it is easy and relatively inexpensive to generate and store mutant plants. Therefore, research on plant ABC transporters will contribute indirectly to developing methods to overcome drug resistance and obesity.

Lead (Pb)- and cadmium (Cd)-tolerant *Arabidopsis* plants that accumulate both Pb and Cd and still grow better than wild-type plants have been engineered (Song et al., 2003). *YCF1*, a yeast gene encoding a vacuolar membrane protein that removes glutathione-conjugated cadmium from the cytoplasm and sequesters it in the vacuole, was introduced into poplar (Shim et al., 2013). The transgenic poplar plants grew better than the wild type in mine tailing soil contaminated with Pb, Cd, and As, and had enhanced extraction capacities for toxic metals and metalloids. Therefore, these plants can be used to clean/stabilize environments polluted with such contaminants.

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REFERENCES

Akiyama, K., Matsuzaki, K., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435**:824–827.

Almen, M.S., Nordstrom, K.J., Fredriksson, R., and Schioth, H.B. (2009). Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biol. **7**:50.

Awai, K., Xu, C., Lu, B., and Benning, C. (2006). Lipid trafficking between the endoplasmic reticulum and the chloroplast. Biochem. Soc. Trans. **34**:395–398.

Banasiak, J., Biała, W., Staszków, A., Swarcewicz, B., Kępczyñska, E., Figlerowicz, M., and Jasiñski, M. (2013). A *Medicago truncatula* ABC transporter belonging to subfamily G modulates the level of isoflavonoids. J. Exp. Bot. **64**:1005–1015.

Banks, J.A., Nishiyama, T., Hasebe, M., Bowman, J.L., Gribskov, M., dePamphilis, C., Albert, V.A., Aono, N., Aoyama, T., Ambrose, B.A., et al. (2011). The *Selaginella* genome identifies genetic changes associated with the evolution of vascular plants. Science **332**:960–963.

Barthlott, W., and Neinhuis, C. (1997). Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta **202**:1–8.

Baxter, I., Tchieu, J., Sussman, M.R., Boutry, M., Palmgren, M.G., Gribskov, M., Harper, J.F., and Axelsen, K.B. (2003). Genomic comparison of P-type ATPase ion pumps in *Arabidopsis* and rice. Plant Physiol. **132**:618–628.

Bessire, M., Borel, S., Fabre, G., Carraça, L., Efremova, N., Yephremov, A., Cao, Y., Jetter, R., Jacquat, A.-C., and Métraux, J.-P. (2011). A member of the PLEIOTROPIC DRUG RESISTANCE family of ATP binding cassette transporters is required for the formation of a functional cuticle in *Arabidopsis*. Plant Cell **23**:1958–1970.

Bienert, M.D., Siegmund, S.E., Drozak, A., Trombik, T., Bultreys, A., Baldwin, I.T., and Boutry, M. (2012). A pleiotropic drug resistance transporter in *Nicotiana tabacum* is involved in defense against the herbivore *Manduca sexta*. Plant J. **72**:745–757.

Bird, D., Beisson, F., Brigham, A., Shin, J., Greer, S., Jetter, R., Kunst, L., Wu, X., Yephremov, A., and Samuels, L. (2007). Characterization of *Arabidopsis* ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. Plant J. **52**:485–498.

Bishopp, A., Lehesranta, S., Vatén, A., El-Showk, S., Scheres, B., Helariutta, K., Mähönen, A.P., Sakakibara, H., and Helariutta, Y. (2011). Phloem-transported cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. Curr. Biol. **21**:927–932.

Blaby, I.K., Blaby-Haas, C.E., Tourasse, N., Hom, E.F., Lopez, D., Aksoy, M., Grossman, A., Umen, J., Dutcher, S., and Porter, M. (2014). The *Chlamydomonas* genome project: a decade on. Trends Plant Sci. **19**:672–680.

Blakeslee, J.J., Bandyopadhyay, A., Lee, O.R., Mravec, J., Titapiwatanakun, B., Sauer, M., Makam, S.N., Cheng, Y., Bouchard, R., Adamec, J., et al. (2007). Interactions among PIN-FORMED and P-glycoprotein auxin transporters in *Arabidopsis*. Plant Cell **19**:131–147.

Bonaventure, G., Beisson, F., Ohlrogge, J., and Pollard, M. (2004). Analysis of the aliphatic monomer composition of polyesters associated with *Arabidopsis* epidermis: occurrence of octadeca-cis-6, cis-9-diene-1,18-dioate as the major component. Plant J. **40**:920–930.

Brohee, S., Barriot, R., Moreau, Y., and Andre, B. (2010). YTPdb: a wiki database of yeast membrane transporters. Biochim. Biophys. Acta **1798**:1908–1912.

Brunetti, P., Zanella, L., De Paolis, A., Di Litta, D., Cecchetti, V., Falasca, G., Barbieri, M., Altamura, M.M., Costantino, P., and Cardarelli, M. (2015). Cadmium-inducible expression of the ABC-type transporter AtABCC3 increases phytochelatin-mediated cadmium tolerance in *Arabidopsis*. J. Exp. Bot. **66**:3815–3829.

Buda, G.J., Barnes, W.J., Fich, E.A., Park, S., Yeats, T.H., Zhao, L., Domozych, D.S., and Rose, J.K. (2013). An ATP binding cassette transporter is required for cuticular wax deposition and desiccation tolerance in the moss *Physcomitrella patens*. Plant Cell **25**:4000–4013.

Bult, C.J., Eppig, J.T., Blake, J.A., Kadin, J.A., Richardson, J.E., and Group, M.G.D. (2016). Mouse genome database 2016. Nucleic Acids Res. **44**:D840–D847.

Burghardt, M., and Riederer, M. (2008). Cuticular transpiration. Annu. Plant Rev. Biol. Plant Cuticle **23**:10.

Campbell, E.J., Schenk, P.M., Kazan, K., Penninckx, I.A., Anderson, J.P., Maclean, D.J., Cammue, B.P., Ebert, P.R., and Manners, J.M. (2003). Pathogen-responsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is regulated by multiple defense signaling pathways in *Arabidopsis*. Plant Physiol. **133**:1272–1284.

Cecchetti, V., Brunetti, P., Napoli, N., Fattorini, L., Altamura, M.M., Costantino, P., and Cardarelli, M. (2015). ABCB1 and ABCB19 auxin transporters have synergistic effects on early and late *Arabidopsis* anther development. J. Integr. Plant Biol. **57**:1089–1098.

Champagne, A., and Boutry, M. (2013). Proteomic snapshot of spearmint (*Mentha spicata* L.) leaf trichomes: a genuine terpenoid factory. Proteomics **13**:3327–3332.

Cho, M., and Cho, H. (2013). The function of ABCB transporters in auxin transport. Plant Signal. Behav. **8**:642–654.

Cho, M., Lee, S.H., and Cho, H.-T. (2007). P-Glycoprotein4 displays auxin efflux transporter–like action in *Arabidopsis* root hair cells and tobacco cells. Plant Cell **19**:3930–3943.

Choi, H., Ohyama, K., Kim, Y.-Y., Jin, J.-Y., Lee, S.B., Yamaoka, Y., Muranaka, T., Suh, M.C., Fujioka, S., and Lee, Y. (2014). The role of *Arabidopsis* ABCG9 and ABCG31 ATP binding cassette transporters in pollen fitness and the deposition of steryl glycosides on the pollen coat. Plant Cell **26**:310–324.

Christie, K.R., Hong, E.L., and Cherry, J.M. (2009). Functional annotations for the *Saccharomyces cerevisiae* genome: the knowns and the known unknowns. Trends Microbiol. **17**:286–294.

Clark, A.G., Eisen, M.B., Smith, D.R., Bergman, C.M., Oliver, B., Markow, T.A., Kaufman, T.C., Kellis, M., Gelbart, W., and Iyer, V.N. (2007). Evolution of genes and genomes on the *Drosophila* phylogeny. Nature **450**:203–218.

Clay, N.K., Adio, A.M., Denoux, C., Jander, G., and Ausubel, F.M. (2009). Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science **323**:95–101.

Crouzet, J., Roland, J., Peeters, E., Trombik, T., Ducos, E., Nader, J., and Boutry, M. (2013). NtPDR1, a plasma membrane ABC transporter from *Nicotiana tabacum*, is involved in diterpene transport. Plant Mol. Biol. **82**:181–192.

De Hertogh, B., Carvajal, E., Talla, E., Dujon, B., Baret, P., and Goffeau, A. (2002). Phylogenetic classification of transporters and other membrane proteins from *Saccharomyces cerevisiae*. Funct. Integr. Genomics **2**:154–170.

De Michele, R., Loque, D., Lalonde, S., and Frommer, W.B. (2012). Ammonium and urea transporter inventory of the *Selaginella* and *Physcomitrella* genomes. Front. Plant Sci. **3**:62.

Dean, J.V., and Mills, J.D. (2004). Uptake of salicylic acid 2-O-beta-D-glucose into soybean tonoplast vesicles by an ATP-binding cassette transporter-type mechanism. Physiol. Plant **120**:603–612.

Dean, M., and Annilo, T. (2005). Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu. Rev. Genomics Hum. Genet. **6**:123–142.

Endo, A., Sawada, Y., Takahashi, H., Okamoto, M., Ikegami, K., Koiwai, H., Seo, M., Toyomasu, T., Mitsuhashi, W., and Shinozaki, K. (2008). Drought induction of *Arabidopsis* 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. Plant Physiol. **147**:1984–1993.

Everant-Bourbouloux, A. (1982). Transport and metabolism of labelled abscisic acid in broad-bean plants (*Vicia faba* L.). Physiol. Plant **54**:431–439.

Ezkurdia, I., Juan, D., Rodriguez, J.M., Frankish, A., Diekhans, M., Harrow, J., Vazquez, J., Valencia, A., and Tress, M.L. (2014). Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes. Hum. Mol. Genet. **23**:5866–5878.

Fan, J., Zhai, Z., Yan, C., and Xu, C. (2015). *Arabidopsis* TRIGALACTOSYLDIACYLGLYCEROL5 interacts with TGD1, TGD2, and TGD4 to facilitate lipid transfer from the endoplasmic reticulum to plastids. Plant Cell **27**:2941–2955.

Footitt, S., Slocombe, S.P., Larner, V., Kurup, S., Wu, Y., Larson, T., Graham, I., Baker, A., and Holdsworth, M. (2002). Control of germination and lipid mobilization by COMATOSE, the *Arabidopsis* homologue of human ALDP. EMBO J. **21**:2912–2922.

Fourcroy, P., Sisó-Terraza, P., Sudre, D., Savirón, M., Reyt, G., Gaymard, F., Abadía, A., Abadia, J., Álvarez-Fernández, A., and Briat, J.F. (2014). Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by *Arabidopsis* roots in response to iron deficiency. New Phytol. **201**:155–167.

Francisco, R.M., Regalado, A., Ageorges, A., Burla, B.J., Bassin, B., Eisenach, C., Zarrouk, O., Vialet, S., Marlin, T., and Chaves, M.M. (2013). ABCC1, an ATP binding cassette protein from grape berry, transports anthocyanidin 3-O-glucosides. Plant Cell **25**:1840–1854.

Frangne, N., Eggmann, T., Koblischke, C., Weissenbock, G., Martinoia, E., and Klein, M. (2002). Flavone glucoside uptake into barley mesophyll and *Arabidopsis* cell culture vacuoles. Energization occurs by H(+)-antiport and ATP-binding cassette-type mechanisms. Plant Physiol. **128**:726–733.

Freeman, B.C., and Beattie, G.A. (2008). An overview of plant defenses against pathogens and herbivores. Plant Health Instructor http://dx.doi.org/10.1094/PHI-I-2008-0226-01.

Fulda, M., Schnurr, J., Abbadi, A., and Heinz, E. (2004). Peroxisomal Acyl-CoA synthetase activity is essential for seedling development in *Arabidopsis thaliana*. Plant Cell **16**:394–405.

Gaedeke, N., Klein, M., Kolukisaoglu, U., Forestier, C., Muller, A., Ansorge, M., Becker, D., Mamnun, Y., Kuchler, K., Schulz, B., et al. (2001). The *Arabidopsis thaliana* ABC transporter AtMRP5 controls root development and stomata movement. EMBO J. **20**:1875–1887.

Gaillard, C., Dufaud, A., Tommasini, R., Kreuz, K., Amrhein, N., and Martinoia, E. (1994). A herbicide antidote (safener) induces the activity of both the herbicide detoxifying enzyme and of a vacuolar transporter for the detoxified herbicide. FEBS Lett. **352**:219–221.

Geisler, M., Blakeslee, J.J., Bouchard, R., Lee, O.R., Vincenzetti, V., Bandyopadhyay, A., Titapiwatanakun, B., Peer, W.A., Bailly, A., Richards, E.L., et al. (2005). Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant J. **44**:179–194.

Gomez-Roldan, V., Fermas, S., Brewer, P.B., Puech-Pages, V., Dun, E.A., Pillot, J.P., Letisse, F., Matusova, R., Danoun, S., Portais, J.C., et al. (2008). Strigolactone inhibition of shoot branching. Nature **455**:189–194.

Goodman, C.D., Casati, P., and Walbot, V. (2004). A multidrug resistance–associated protein involved in anthocyanin transport in *Zea mays*. Plant Cell **16**:1812–1826.

Hayashi, M., Nito, K., Takei-Hoshi, R., Yagi, M., Kondo, M., Suenaga, A., Yamaya, T., and Nishimura, M. (2002). Ped3p is a peroxisomal ATP-binding cassette transporter that might supply substrates for fatty acid β-oxidation. Plant Cell Physiol. **43**:1–11.

Heredia, A. (2003). Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer. Biochim. Biophys. Acta **1620**:1–7.

Hillier, L.W., Coulson, A., Murray, J.I., Bao, Z., Sulston, J.E., and Waterston, R.H. (2005). Genomics in *C. elegans*: so many genes, such a little worm. Genome Res. **15**:1651–1660.

Huang, C.F., Yamaji, N., Mitani, N., Yano, M., Nagamura, Y., and Ma, J.F. (2009). A bacterial-type ABC transporter is involved in aluminum tolerance in rice. Plant Cell **21**:655–667.

Ikegami, K., Okamoto, M., Seo, M., and Koshiba, T. (2009). Activation of abscisic acid biosynthesis in the leaves of *Arabidopsis thaliana* in response to water deficit. J. Plant Res. **122**:235–243.

Ito, H., and Gray, W.M. (2006). A gain-of-function mutation in the *Arabidopsis* pleiotropic drug resistance transporter PDR9 confers resistance to auxinic herbicides. Plant Physiol. **142**:63–74.

James, C.N., Horn, P.J., Case, C.R., Gidda, S.K., Zhang, D., Mullen, R.T., Dyer, J.M., Anderson, R.G., and Chapman, K.D. (2010). Disruption of the *Arabidopsis* CGI-58 homologue produces Chanarin-Dorfman-like lipid droplet accumulation in plants. Proc. Natl. Acad. Sci. USA **107**:17833–17838.

Jasiński, M., Stukkens, Y., Degand, H., Purnelle, B., Marchand-Brynaert, J., and Boutry, M. (2001). A plant plasma membrane ATP binding cassette–type transporter is involved in antifungal terpenoid secretion. Plant Cell **13**:1095–1107.

Jetter, R., Kunst, L., and Samuels, A.L. (2008). 4 Composition of plant cuticular waxes. Annu. Plant Rev. Biol. Plant Cuticle **23**:145.

Ji, H., Peng, Y., Meckes, N., Allen, S., Stewart, C.N., and Traw, M.B. (2014). ATP-dependent binding cassette transporter G family

member 16 increases plant tolerance to abscisic acid and assists in basal resistance against pseudomonas syringae DC3000. Plant Physiol. **166**:879–888.

Kamimoto, Y., Terasaka, K., Hamamoto, M., Takanashi, K., Fukuda, S., Shitan, N., Sugiyama, A., Suzuki, H., Shibata, D., and Wang, B. (2012). *Arabidopsis* ABCB21 is a facultative auxin importer/exporter regulated by cytoplasmic auxin concentration. Plant Cell Physiol. **53**:2090–2100.

Kaneda, M., Schuetz, M., Lin, B.S., Chanis, C., Hamberger, B., Western, T.L., Ehlting, J., and Samuels, A.L. (2011). ABC transporters coordinately expressed during lignification of *Arabidopsis* stems include a set of ABCBs associated with auxin transport. J. Exp. Bot. **62**:2063–2077.

Kang, J., Hwang, J.-U., Lee, M., Kim, Y.-Y., Assmann, S.M., Martinoia, E., and Lee, Y. (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc. Natl. Acad. Sci. USA **107**:2355–2360.

Kang, J., Park, J., Choi, H., Burla, B., Kretzschmar, T., Lee, Y., and Martinoia, E. (2011). Plant ABC transporters. Arabidopsis Book **9**:e0153.

Kang, J., Yim, S., Choi, H., Kim, A., Lee, K.P., Lopez-Molina, L., Martinoia, E., and Lee, Y. (2015). Abscisic acid transporters cooperate to control seed germination. Nat. Commun. **6**:8113.

Kanno, Y., Jikumaru, Y., Hanada, A., Nambara, E., Abrams, S.R., Kamiya, Y., and Seo, M. (2010). Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. Plant Cell Physiol. **51**:1988–2001.

Kanno, Y., Hanada, A., Chiba, Y., Ichikawa, T., Nakazawa, M., Matsui, M., Koshiba, T., Kamiya, Y., and Seo, M. (2012). Identification of an abscisic acid transporter by functional screening using the receptor complex as a sensor. Proc. Natl. Acad. Sci. USA **109**:9653–9658.

Kiba, T., Takei, K., Kojima, M., and Sakakibara, H. (2013). Side-chain modification of cytokinins control shoot growth in *Arabidopsis*. Dev. Cell **27**:452–461.

Kim, D.Y., Bovet, L., Maeshima, M., Martinoia, E., and Lee, Y. (2007). The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J. **50**:207–218.

Kim, D.Y., Jin, J.Y., Alejandro, S., Martinoia, E., and Lee, Y. (2010). Overexpression of AtABCG36 improves drought and salt stress resistance in *Arabidopsis*. Physiol. Plant **139**:170–180.

Kim, S., Yamaoka, Y., Ono, H., Kim, H., Shim, D., Maeshima, M., Martinoia, E., Cahoon, E.B., Nishida, I., and Lee, Y. (2013). AtABCA9 transporter supplies fatty acids for lipid synthesis to the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **110**:773–778.

Klein, M., Martinoia, E., and Weissenbock, G. (1998). Directly energized uptake of beta-estradiol 17-(beta-D-glucuronide) in plant vacuoles is strongly stimulated by glutathione conjugates. J. Biol. Chem. **273**:262–270.

Klein, M., Perfus-Barbeoch, L., Frelet, A., Gaedeke, N., Reinhardt, D., Mueller-Roeber, B., Martinoia, E., and Forestier, C. (2003). The plant multidrug resistance ABC transporter AtMRP5 is involved in guard cell hormonal signalling and water use. Plant J. **33**:119–129.

Ko, D., Kang, J., Kiba, T., Park, J., Kojima, M., Do, J., Kim, K.Y., Kwon, M., Endler, A., Song, W.-Y., et al. (2014). *Arabidopsis* ABCG14 is essential for the root-to-shoot translocation of cytokinin. Proc. Natl. Acad. Sci. USA **111**:7150–7155.

Krattinger, S.G., Lagudah, E.S., Spielmeyer, W., Singh, R.P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L.L., and Keller, B. (2009). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science **323**:1360–1363.

Krauss, P., Markstädter, C., and Riederer, M. (1997). Attenuation of UV radiation by plant cuticles from woody species. Plant Cell Environ. **20**:1079–1085.

Kretzschmar, T., Kohlen, W., Sasse, J., Borghi, L., Schlegel, M., Bachelier, J.B., Reinhardt, D., Bours, R., Bouwmeester, H.J., and Martinoia, E. (2012). A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. Nature **483**:341–344.

Kreuz, K., Tommasini, R., and Martinoia, E. (1996). Old enzymes for a new job (herbicide detoxification in plants). Plant Physiol. **111**:349–353.

Kunst, L., and Samuels, A.L. (2003). Biosynthesis and secretion of plant cuticular wax. Prog. Lipid Res. **42**:51–80.

Kuromori, T., Miyaji, T., Yabuuchi, H., Shimizu, H., Sugimoto, E., Kamiya, A., Moriyama, Y., and Shinozaki, K. (2010). ABC transporter AtABCG25 is involved in abscisic acid transport and responses. Proc. Natl. Acad. Sci. USA **107**:2361–2366.

Kuromori, T., Sugimoto, E., and Shinozaki, K. (2014). Intertissue signal transfer of abscisic acid from vascular cells to guard cells. Plant Physiol. **164**:1587–1592.

Lamesch, P., Berardini, T.Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D.L., and Garcia-Hernandez, M. (2012). The Arabidopsis information resource (TAIR): improved gene annotation and new tools. Nucleic Acids Res. **40**:D1202–D1210.

Lang, D., Zimmer, A.D., Rensing, S.A., and Reski, R. (2008). Exploring plant biodiversity: the *Physcomitrella* genome and beyond. Trends Plant Sci. **13**:542–549.

Larsen, P.B., Kochian, L.V., and Howell, S.H. (1997). Al inhibits both shoot development and root growth in als3, an Al-sensitive *Arabidopsis* mutant. Plant Physiol. **114**:1207–1214.

Larsen, P.B., Geisler, M.J., Jones, C.A., Williams, K.M., and Cancel, J.D. (2005). ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*. Plant J. **41**:353–363.

Lee, S.C., and Luan, S. (2012). ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant. Cell Environ. **35**:53–60.

Lee, M., Choi, Y., Burla, B., Kim, Y.-Y., Jeon, B., Maeshima, M., Yoo, J.-Y., Martinoia, E., and Lee, Y. (2008). The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO_2. Nat. Cell Biol. **10**:1217–1223.

Lewis, D.R., Wu, G., Ljung, K., and Spalding, E.P. (2009). Auxin transport into cotyledons and cotyledon growth depend similarly on the ABCB19 Multidrug Resistance-like transporter. Plant J. **60**:91–101.

Lousa, C.D.M., van Roermund, C.W., Postis, V.L., Dietrich, D., Kerr, I.D., Wanders, R.J., Baldwin, S.A., Baker, A., and Theodoulou, F.L. (2013). Intrinsic acyl-CoA thioesterase activity of a peroxisomal ATP binding cassette transporter is required for transport and metabolism of fatty acids. Proc. Natl. Acad. Sci. USA **110**:1279–1284.

Lu, B., and Benning, C. (2009). A 25-amino acid sequence of the *Arabidopsis* TGD2 protein is sufficient for specific binding of phosphatidic acid. J. Biol. Chem. **284**:17420–17427.

Lu, Y.P., Li, Z.S., and Rea, P.A. (1997). AtMRP1 gene of *Arabidopsis* encodes a glutathione S-conjugate pump: isolation and functional definition of a plant ATP-binding cassette transporter gene. Proc. Natl. Acad. Sci. USA **94**:8243–8248.

Lu, B., Xu, C., Awai, K., Jones, A.D., and Benning, C. (2007). A small ATPase protein of *Arabidopsis*, TGD3, involved in chloroplast lipid import. J. Biol. Chem. **282**:35945–35953.

Lu, X., Dittgen, J., Piœlewska-Bednarek, M., Molina, A., Schneider, B., Svatoš, A., Doubský, J., Schneeberger, K., Weigel, D., and Bednarek, P. (2015). Mutant allele-specific uncoupling of PEN3

functions reveals engagement of the ABC transporter in distinct tryptophan metabolic pathways. Plant Physiol. **168**:814–827.

Luo, B., Xue, X.Y., Hu, W.L., Wang, L.J., and Chen, X.Y. (2007). An ABC transporter gene of *Arabidopsis thaliana*, AtWBC11, is involved in cuticle development and prevention of organ fusion. Plant Cell Physiol. **48**:1790–1802.

McFarlane, H.E., Shin, J.J., Bird, D.A., and Samuels, A.L. (2010). *Arabidopsis* ABCG transporters, which are required for export of diverse cuticular lipids, dimerize in different combinations. Plant Cell **22**:3066–3075.

Merchant, S.S., Prochnik, S.E., Vallon, O., Harris, E.H., Karpowicz, S.J., Witman, G.B., Terry, A., Salamov, A., Fritz-Laylin, L.K., Marechal-Drouard, L., et al. (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science **318**:245–250.

Merilo, E., Jalakas, P., Laanemets, K., Mohammadi, O., Horak, H., Kollist, H., and Brosche, M. (2015). Abscisic acid transport and homeostasis in the context of stomatal regulation. Mol. Plant **8**:1321–1333.

Müller, C. (2008). 13 Plant–Insect interactions on cuticular surfaces. Annu. Plant Rev. Biol. Plant Cuticle **23**:398.

Mutch, D.M., Anderle, P., Fiaux, M., Mansourian, R., Vidal, K., Wahli, W., Williamson, G., and Roberts, M.A. (2004). Regional variations in ABC transporter expression along the mouse intestinal tract. Physiol. Genomics **17**:11–20.

Nagy, R., Grob, H., Weder, B., Green, P., Klein, M., Frelet-Barrand, A., Schjoerring, J.K., Brearley, C., and Martinoia, E. (2009). The *Arabidopsis* ATP-binding Cassette Protein AtMRP5/AtABCC5 is a high affinity inositol hexakisphosphate transporter involved in guard cell signaling and phytate storage. J. Biol. Chem. **284**:33614–33622.

Nambara, E., and Marion-Poll, A. (2005). Abscisic acid biosynthesis and catabolism. Annu. Rev. Plant Biol. **56**:165–185.

Nawrath, C. (2002). The biopolymers cutin and suberin. Arabidopsis Book **1**:e0021.

Nguyen, V.N., Moon, S., and Jung, K.H. (2014). Genome-wide expression analysis of rice ABC transporter family across spatio-temporal samples and in response to abiotic stresses. J. Plant Physiol. **171**:1276–1288.

Niu, B.X., He, F.R., He, M., Ren, D., Chen, L.T., and Liu, Y.G. (2013). The ATP-binding cassette transporter OsABCG15 is required for anther development and pollen fertility in rice. J. Integr. Plant Biol. **55**:710–720.

Nyathi, Y., Lousa, C.D.M., Van Roermund, C.W., Wanders, R.J., Johnson, B., Baldwin, S.A., Theodoulou, F.L., and Baker, A. (2010). The *Arabidopsis* peroxisomal ABC transporter, comatose, complements the *Saccharomyces cerevisiae* pxa1 pxa2Δ mutant for metabolism of long-chain fatty acids and exhibits fatty acyl-CoA-stimulated ATPase activity. J. Biol. Chem. **285**:29892–29902.

Ouyang, S., Zhu, W., Hamilton, J., Lin, H., Campbell, M., Childs, K., Thibaud-Nissen, F., Malek, R.L., Lee, Y., and Zheng, L. (2007). The TIGR rice genome annotation resource: improvements and new features. Nucleic Acids Res. **35**:D883–D887.

Panikashvili, D., Savaldi-Goldstein, S., Mandel, T., Yifhar, T., Franke, R.B., Hofer, R., Schreiber, L., Chory, J., and Aharoni, A. (2007). The *Arabidopsis* DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. Plant Physiol. **145**:1345–1360.

Panikashvili, D., Shi, J.X., Bocobza, S., Franke, R.B., Schreiber, L., and Aharoni, A. (2010). The *Arabidopsis* DSO/ABCG11 transporter affects cutin metabolism in reproductive organs and suberin in roots. Mol. Plant **3**:563–575.

Panikashvili, D., Shi, J.X., Schreiber, L., and Aharoni, A. (2011). The *Arabidopsis* ABCG13 transporter is required for flower cuticle secretion and patterning of the petal epidermis. New Phytol. **190**:113–124.

Park, J., Song, W.Y., Ko, D., Eom, Y., Hansen, T.H., Schiller, M., Lee, T.G., Martinoia, E., and Lee, Y. (2012). The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. Plant J. **69**:278–288.

Park, S., Gidda, S.K., James, C.N., Horn, P.J., Khuu, N., Seay, D.C., Keereetaweep, J., Chapman, K.D., Mullen, R.T., and Dyer, J.M. (2013). The alpha/beta hydrolase CGI-58 and peroxisomal transport protein PXA1 coregulate lipid homeostasis and signaling in *Arabidopsis*. Plant Cell **25**:1726–1739.

Paumi, C.M., Chuk, M., Snider, J., Stagljar, I., and Michaelis, S. (2009). ABC transporters in *Saccharomyces cerevisiae* and their interactors: new technology advances the biology of the ABCC (MRP) subfamily. Microbiol. Mol. Biol. Rev. **73**:577–593.

Piecuch, A., and Oblak, E. (2014). Yeast ABC proteins involved in multidrug resistance. Cell Mol. Biol. Lett. **19**:1–22.

Pighin, J.A., Zheng, H., Balakshin, L.J., Goodman, I.P., Western, T.L., Jetter, R., Kunst, L., and Samuels, A.L. (2004). Plant cuticular lipid export requires an ABC transporter. Science **306**:702–704.

Prasad, R., and Goffeau, A. (2012). Yeast ATP-binding cassette transporters conferring multidrug resistance. Annu. Rev. Microbiol. **66**:39–63.

Procko, E., O'Mara, M.L., Bennett, W.D., Tieleman, D.P., and Gaudet, R. (2009). The mechanism of ABC transporters: general lessons from structural and functional studies of an antigenic peptide transporter. FASEB J. **23**:1287–1302.

Quilichini, T.D., Douglas, C.J., and Samuels, A.L. (2014). New views of tapetum ultrastructure and pollen exine development in *Arabidopsis thaliana*. Ann. Bot. **114**:1189–1201.

Ren, Q., Chen, K., and Paulsen, I.T. (2007). TransportDB: a comprehensive database resource for cytoplasmic membrane transport systems and outer membrane channels. Nucleic Acids Res. **35**:D274–D279.

Roston, R.L., Gao, J., Murcha, M.W., Whelan, J., and Benning, C. (2012). TGD1, -2, and -3 proteins involved in lipid trafficking form ATP-binding cassette (ABC) transporter with multiple substrate-binding proteins. J. Biol. Chem. **287**:21406–21415.

Ruocco, M., Ambrosino, P., Lanzuise, S., Woo, S.L., Lorito, M., and Scala, F. (2011). Four potato (*Solanum tuberosum*) ABCG transporters and their expression in response to abiotic factors and *Phytophthora infestans* infection. J. Plant Physiol. **168**:2225–2233.

Russell, L., Larner, V., Kurup, S., Bougourd, S., and Holdsworth, M. (2000). The *Arabidopsis* COMATOSE locus regulates germination potential. Development **127**:3759–3767.

Ruzicka, K., Strader, L.C., Bailly, A., Yang, H., Blakeslee, J., Langowski, L., Nejedla, E., Fujita, H., Itoh, H., Syono, K., et al. (2010). *Arabidopsis* PIS1 encodes the ABCG37 transporter of auxinic compounds including the auxin precursor indole-3-butyric acid. Proc. Natl. Acad. Sci. USA **107**:10749–10753.

Sanchez-Fernandez, R., Davies, T.G., Coleman, J.O., and Rea, P.A. (2001). The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. J. Biol. Chem. **276**:30231–30244.

Sasse, J., Simon, S., Gubeli, C., Liu, G.W., Cheng, X., Friml, J., Bouwmeester, H., Martinoia, E., and Borghi, L. (2015). Asymmetric localizations of the ABC transporter PaPDR1 trace paths of directional strigolactone transport. Curr. Biol. **25**:647–655.

Seo, M., and Koshiba, T. (2011). Transport of ABA from the site of biosynthesis to the site of action. J. Plant Res. **124**:501–507.

Seto, Y., and Yamaguchi, S. (2014). Strigolactone biosynthesis and perception. Curr. Opin. Plant Biol. **21**:1–6.

Sheps, J.A., Ralph, S., Zhao, Z., Baillie, D.L., and Ling, V. (2004). The ABC transporter gene family of *Caenorhabditis elegans* has

implications for the evolutionary dynamics of multidrug resistance in eukaryotes. Genome Biol. **5**:R15.

Shi, J., Wang, H., Schellin, K., Li, B., Faller, M., Stoop, J.M., Meeley, R.B., Ertl, D.S., Ranch, J.P., and Glassman, K. (2007). Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. Nat. Biotechnol. **25**:930–937.

Shim, D., Kim, S., Choi, Y.-I., Song, W.-Y., Park, J., Youk, E.S., Jeong, S.-C., Martinoia, E., Noh, E.-W., and Lee, Y. (2013). Transgenic poplar trees expressing yeast cadmium factor 1 exhibit the characteristics necessary for the phytoremediation of mine tailing soil. Chemosphere **90**:1478–1486.

Shitan, N., Bazin, I., Dan, K., Obata, K., Kigawa, K., Ueda, K., Sato, F., Forestier, C., and Yazaki, K. (2003). Involvement of CjMDR1, a plant multidrug-resistance-type ATP-binding cassette protein, in alkaloid transport in *Coptis japonica*. Proc. Natl. Acad. Sci. USA **100**:751–756.

Song, W.-Y., Sohn, E.J., Martinoia, E., Lee, Y.J., Yang, Y.-Y., Jasinski, M., Forestier, C., Hwang, I., and Lee, Y. (2003). Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat. Biotechnol. **21**:914–919.

Song, W.Y., Park, J., Mendoza-Cozatl, D.G., Suter-Grotemeyer, M., Shim, D., Hortensteiner, S., Geisler, M., Weder, B., Rea, P.A., Rentsch, D., et al. (2010). Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. Proc. Natl. Acad. Sci. USA **107**:21187–21192.

Song, W.Y., Yamaki, T., Yamaji, N., Ko, D., Jung, K.H., Fujii-Kashino, M., An, G., Martinoia, E., Lee, Y., and Ma, J.F. (2014). A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. Proc. Natl. Acad. Sci. USA **111**:15699–15704.

Stein, M., Dittgen, J., Sánchez-Rodríguez, C., Hou, B.-H., Molina, A., Schulze-Lefert, P., Lipka, V., and Somerville, S. (2006). *Arabidopsis* PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell **18**:731–746.

Strader, L.C., and Bartel, B. (2009). The *Arabidopsis* PLEIOTROPIC DRUG RESISTANCE8/ABCG36 ATP binding cassette transporter modulates sensitivity to the auxin precursor indole-3-butyric acid. Plant Cell **21**:1992–2007.

Strader, L.C., and Bartel, B. (2011). Transport and metabolism of the endogenous auxin precursor indole-3-butyric acid. Mol. Plant **4**:477–486.

Stukkens, Y., Bultreys, A., Grec, S., Trombik, T., Vanham, D., and Boutry, M. (2005). NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from *Nicotiana plumbaginifolia*, plays a major role in plant pathogen defense. Plant Physiol. **139**:341–352.

Suh, M.C., Samuels, A.L., Jetter, R., Kunst, L., Pollard, M., Ohlrogge, J., and Beisson, F. (2005). Cuticular lipid composition, surface structure, and gene expression in *Arabidopsis* stem epidermis. Plant Physiol. **139**:1649–1665.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. **30**:2725–2729.

Terasaka, K., Blakeslee, J.J., Titapiwatanakun, B., Peer, W.A., Bandyopadhyay, A., Makam, S.N., Lee, O.R., Richards, E.L., Murphy, A.S., and Sato, F. (2005). PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. Plant Cell **17**:2922–2939.

Treutter, D. (2006). Significance of flavonoids in plant resistance: a review. Environ. Chem. Lett. **4**:147–157.

Ukitsu, H., Kuromori, T., Toyooka, K., Goto, Y., Matsuoka, K., Sakuradani, E., Shimizu, S., Kamiya, A., Imura, Y., Yuguchi, M., et al. (2007). Cytological and biochemical analysis of COF1, an *Arabidopsis* mutant of an ABC transporter gene. Plant Cell Physiol. **48**:1524–1533.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., et al. (2008). Inhibition of shoot branching by new terpenoid plant hormones. Nature **455**:195–200.

Underwood, W., and Somerville, S.C. (2013). Perception of conserved pathogen elicitors at the plasma membrane leads to relocalization of the *Arabidopsis* PEN3 transporter. Proc. Natl. Acad. Sci. USA **110**:12492–12497.

Van Belle, D., and Andre, B. (2001). A genomic view of yeast membrane transporters. Curr. Opin. Cell Biol. **13**:389–398.

Van Den Brûle, S., Müller, A., Fleming, A.J., and Smart, C.C. (2002). The ABC transporter SpTUR2 confers resistance to the antifungal diterpene sclareol. Plant J. **30**:649–662.

Vasiliou, V., Vasiliou, K., and Nebert, D.W. (2009). Human ATP-binding cassette (ABC) transporter family. Hum. Genomics **3**:281.

Verrier, P.J., Bird, D., Burla, B., Dassa, E., Forestier, C., Geisler, M., Klein, M., Kolukisaoglu, U., Lee, Y., Martinoia, E., et al. (2008). Plant ABC proteins–a unified nomenclature and updated inventory. Trends Plant Sci. **13**:151–159.

Von Uexküll, H., and Mutert, E. (1995). Global extent, development and economic impact of acid soils. Plant Soil **171**:1–15.

Waldie, T., McCulloch, H., and Leyser, O. (2014). Strigolactones and the control of plant development: lessons from shoot branching. Plant J. **79**:607–622.

Wallace, S., Fleming, A., Wellman, C.H., and Beerling, D.J. (2011). Evolutionary development of the plant spore and pollen wall. AoB Plants **2011**:plr027.

Wang, C., Liu, Y., Li, S.-S., and Han, G.-Z. (2015). Insights into the origin and evolution of the plant hormone signaling machinery. Plant Physiol. **167**:872–886.

Wellman, C.H. (2004). Origin, function and development of the spore wall in early land plants. In The Evolution of Plant Physiology. From Whole Plants to Ecosystems. Linnean Society Symposium Series No. 21, A.R. Hemsley and I. Poole, eds. (Amsterdam: Elsevier Academic Press), pp. 43–63.

Wilkens, S. (2015). Structure and mechanism of ABC transporters. F1000Prime Rep. **7**:14.

Wilkinson, S., and Davies, W.J. (1997). Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiol. **113**:559–573.

Wu, L., Guan, Y., Wu, Z., Yang, K., Lv, J., Converse, R., Huang, Y., Mao, J., Zhao, Y., and Wang, Z. (2014). OsABCG15 encodes a membrane protein that plays an important role in anther cuticle and pollen exine formation in rice. Plant Cell Rep. **33**:1881–1899.

Xie, X., Wang, G., Yang, L., Cheng, T., Gao, J., Wu, Y., and Xia, Q. (2015). Cloning and characterization of a novel *Nicotiana tabacum* ABC transporter involved in shoot branching. Physiol. Plant **153**:299–306.

Xu, C., Fan, J., Riekhof, W., Froehlich, J.E., and Benning, C. (2003). A permease-like protein involved in ER to thylakoid lipid transfer in *Arabidopsis*. EMBO J. **22**:2370–2379.

Xu, C., Fan, J., Cornish, A.J., and Benning, C. (2008). Lipid trafficking between the endoplasmic reticulum and the plastid in *Arabidopsis* requires the extraplastidic TGD4 protein. Plant Cell **20**:2190–2204.

Yadav, V., Molina, I., Ranathunge, K., Castillo, I.Q., Rothstein, S.J., and Reed, J.W. (2014). ABCG transporters are required for suberin and pollen wall extracellular barriers in *Arabidopsis*. Plant Cell **26**:3569–3588.

Yang, H., and Murphy, A.S. (2009). Functional expression and characterization of *Arabidopsis* ABCB, AUX 1 and PIN auxin transporters in *Schizosaccharomyces pombe*. Plant J. **59**:179–191.

Yazaki, K., Shitan, N., Takamatsu, H., Ueda, K., and Sato, F. (2001). A novel *Coptis japonica* multidrug-resistant protein preferentially expressed in the alkaloid-accumulating rhizome. J. Exp. Bot. **52**:877–879.

Ye, A.Y., Liu, Q.R., Li, C.Y., Zhao, M., and Qu, H. (2014). Human transporter database: comprehensive knowledge and discovery tools in the human transporter genes. PLoS One **9**:e88883.

Yu, F., and De Luca, V. (2013). ATP-binding cassette transporter controls leaf surface secretion of anticancer drug components in Catharanthus roseus. Proc. Natl. Acad. Sci. USA **110**:15830–15835.

Zhang, H., Zhu, H., Pan, Y., Yu, Y., Luan, S., and Li, L. (2014a). A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates ABA sensitivity and drought tolerance in *Arabidopsis*. Mol. Plant **7**:1522–1532.

Zhang, K., Novak, O., Wei, Z., Gou, M., Zhang, X., Yu, Y., Yang, H., Cai, Y., Strnad, M., and Liu, C.-J. (2014b). Arabidopsis ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins. Nat. Commun. **5**:3274.

Zhao, J. (2015). Flavonoid transport mechanisms: how to go, and with whom. Trends Plant Sci. **20**:576–585.

Zimmer, A.D., Lang, D., Buchta, K., Rombauts, S., Nishiyama, T., Hasebe, M., Van de Peer, Y., Rensing, S.A., and Reski, R. (2013). Reannotation and extended community resources for the genome of the non-seed plant *Physcomitrella patens* provide insights into the evolution of plant gene structures and functions. BMC Genomics **14**:498.

Zolman, B.K., Silva, I.D., and Bartel, B. (2001). The *Arabidopsis* pxa1 mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid β-oxidation. Plant Physiol. **127**:1266–1278.